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L4 11 DUP REM L2 (12 DUPLICATES REMOVED)
L5 6 DUP REM L3 (3 DUPLICATES REMOVED)
L6 7 S L1 (P) (ELISA)
L7 10 S L1 AND (ELISA)
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L4             1 S L3 AND ANTIBOD?

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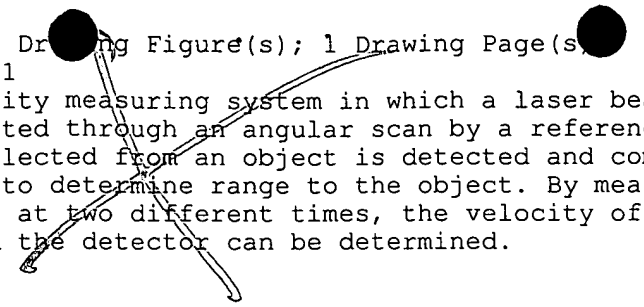
NUMBER OF DRAWINGS: 10 Drawing Figure(s); 1 Drawing Page(s)
LINE COUNT: 291
AB A ranging and velocity measuring system in which a laser beam is periodically deflected through an angular scan by a reference periodic wave and energy reflected from an object is detected and compared with the reference wave to determine range to the object. By measuring the range to the object at two different times, the velocity of the object toward or away from the detector can be determined.



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From: Jamroz, Margaret
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Van den Heuvel MM et al. J Leukoc Biol 1999 Nov;66(5):858-66

Buechler C, et al.J Leukoc Biol 2000 Jan;67(1):97-103

Ritter et al. Pathobiology 1999;67(5-6):257-61

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Pathobiology

Pathobiology 1999;67:257–261

The Scavenger Receptor CD163: Regulation, Promoter Structure and Genomic Organization

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Institute for Clinical Chemistry and Laboratory Medicine, University of Regensburg, Germany

Key Words

CD163 · Monocyte · Cytokine · Inflammation · Promoter

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Introduction

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The first two authors contributed equally to this work.

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dominant form, several variant mRNAs have been described that encode CD163 isoforms with altered cytoplasmic or extracellular domains [1].

Although the precise function of CD163 is still unknown, several observations reveal an important role of this protein in the downregulation of the inflammatory response. Recently Högger et al. [2] demonstrated that CD163 is also recognized by the mAb RM3/1. RM3/1-positive cells develop in vivo during the healing phase and produce anti-inflammatory and angiogenic factors [3]. In contrast, IFN-γ-activated cells, which are present in early inflammation, do not express this antigen. Therefore, it seems that CD163 expression is associated with anti-inflammatory processes and with the downregulation of the inflammatory response.

In order to further characterize the regulation of CD163, we investigated the differentiation-dependent CD163 mRNA and protein expression and the influence of several pro- and anti-inflammtory stimuli on CD163 mRNA and protein in monocytes and macrophages [4]. We also cloned the promoter region of the gene in order to get insight into the transcriptional regulation of the CD163 gene expression. Additional studies of the genomic organization of the CD163 gene revealed the origin of the CD163 variants by alternative splicing [5].

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Peripheral blood monocytes from healthy volunteers were isolated by leukapheresis followed by elutriation. Fractions containing >90% monocyte purity were pooled and cultured on plastic Petri dishes (10^6 cells/ml) in a serum-free macrophage SFM medium supplemented with the indicated stimuli. The following cytokines were used: M-CSF: 5 ng/ml, IL-10: 10 ng/ml, IL-6: 10 ng/ml, IFN-γ: 10 ng/ml, TNF-α: 10 ng/ml, GM-CSF (5 ng/ml) in combination with IL-4 (10 ng/ml). Additional bioactive substances applied were dexamethasone: 40 ng/ml and LPS, *Escherichia coli* serotype 055:B5: 1, 10 or 100 ng/ml in the presence and 1 μg/ml in the absence of serum.

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The CD163 mRNA expression was also investigated in several human and mouse cell lines of monocytic origin, including THP-1, U937, HL-60, P338, J-774A.1, MonoMac6 and RAW264.7. None of these cell lines expresses CD163 mRNA at significant levels even when the cells were differentiated by the addition of PMA. The only cell line, which displayed a high expression of CD163 mRNA, which could even be increased by stimulation with PMA, was the true histocytic lymphoma cell line SU-DHL-1.

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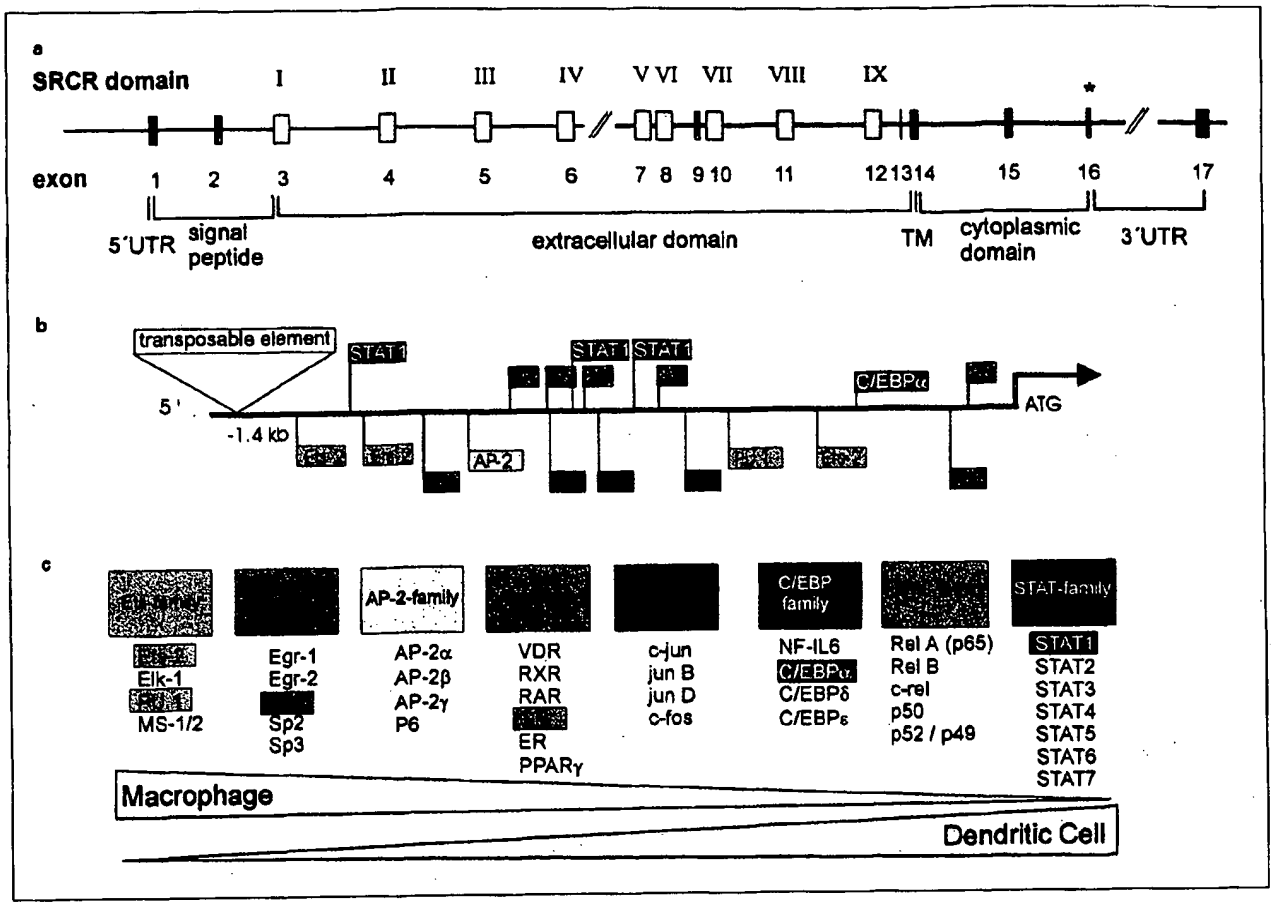
In contrast, stimulation of monocytes or macrophages with proinflammatory molecules like IFN-γ and TNF-α



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results in a suppression of CD163 gene transcription and in a decrease of the cell surface expression of CD163 protein. We also investigated the influence of the most potent inflammatory stimulus, lipopolysaccharide (LPS), on CD163 expression [4]. We found that in the presence of serum 1–10 ng/ml LPS are sufficient to induce a significant downregulation of CD163 mRNA in monocytes and macrophages. The same effect was observed, when monocytes or macrophages were stimulated with LPS in the absence of serum. However, under these conditions, much higher LPS concentrations (1 μg/ml) were necessary to induce a similar downregulation. The cell surface expression of CD163 on monocytes was also found to be downregulated following stimulation with LPS. This LPS-induced downregulation of CD163 may contribute to the absence of CD163-positive cells in early inflammation.

Promoter Structure of the CD163 Gene

In order to further characterize the transcriptional regulation of CD163 expression, we cloned the promoter region of the CD163 gene and determined the transcription initiation sites. Primer extension analysis identified one major transcription start site and six alternative initiation sites, which are used to a lesser extent. The adjacent 5'-flanking region contains several putative binding sites for transcription factors like PU.1, C/EBPα, Ets-2 and AP-1, which have been shown to play an important role in myeloid-specific gene expression (fig. 1b). There

are also potential glucocorticoid receptor binding sites, which might contribute to the strong induction of CD163 transcription following glucocorticoid treatment of monocytes and macrophages. The proximal sequence contains no TATA box, which is a common feature of many myeloid-specific promoter sequences and is in accordance with the observation of multiple transcription initiation sites. We also identified an L1-transposable element located 1.4 kb upstream of the major start site. Currently, we characterize the regulation of the promoter activity by pro- and anti-inflammatory stimuli and the influence of the L1 element.

Genomic Organization, Chromosomal Localization and Splice Variants of CD163

Regarding the number and spacing of cysteine residues per SRCR domain, the SRCR superfamily can be divided into two subfamilies and it has been proposed that these subfamilies differ in their genomic organization. The CD163 gene is composed of 17 exons and 16 introns and spans at least 35 kb (fig. 1a). The extracelluar portion of the mature protein is encoded by exons 1 through 14, with each SRCR domain being encoded by a separate exon. This exon organization is similar to other members of group B subfamily of the SRCR superfamily. While the SRCR domains of group B SRCR proteins seem to be encoded by single exons, SRCR domains of subfamily A proteins seem to be encoded by two exons. This indicates an independent evolution of group A and group B subfamilies.

Using fluorescent in situ hybridization with a fragment of the CD163 gene, which spanned the first three exons and introns, we mapped the CD163 gene to chromosome 12p13 [5]. Rearrangements of the short arm of chromosome 12 are one of the most frequently recurring motifs in a broad range of hematologic malignancies. Whether the CD163 gene is affected by these rearrangements has to be investigated.

Recently, several different forms of the CD163 mRNA have been described [1], which encode CD163 isoforms with altered cytoplasmic or extracellular domains. Information of the exon-intron boundaries of the gene reveals that all of these variants result from alternative splicing. Both described cytoplasmic isoforms arise from alternative splicing of intron 15. Since both variants are found at significant levels, it may be suggested that some kind of splice site regulation is involved in this splicing pattern. Another variant results from the retention of intron 7. The corresponding protein dislays an insertion of 33 aa between SRCR domain five and six. A putative truncated CD163 isoform, which is composed of only three SRCR domains and contains no transmembrane region, arises from alternative splicing of intron 5, which results in a frame shift and a premature termination of translation. However, the functional differences of these isoforms have to be evaluated.

Discussion

CD163 is a member of the SRCR superfamily that is exclusively expressed on monocytic cells. CD163 is abundant on most tissue macrophages, while dendritic cells are not stained with CD163-specific mAbs. We demonstrate that in vitro phagocytic differentiation of monocytes induces CD163 mRNA and protein expression, while dendritic differentiation of these cells suppresses the expression of CD163. Because CD163 expression is associated with the anti-inflammatory response of monocytes and macrophages, we were also interested in the regulation of CD163 by pro- and anti-inflammatory mediators. Our results show that proinflammatory stimuli including the cytokines IFN-γ and TNF-α as well as LPS suppress CD163 mRNA and protein in monocytes and macrophages [4]. The abundance of these stimuli in early inflammation may contribute to the absence of CD163-positive cells in this phase. In contrast, the anti-inflammatory cytokine IL-10 and the glucocorticoid dexamethasone both induce a rapid upregulation of CD163 in monocytes and macrophages. A similar effect was observed for IL-6. Since IL-10 and IL-6 secretion are induced by IFN-γ and LPS, we assume that these cytokines mediate the high expression of CD163 on anti-inflammatory macrophage subsets.

In order to identify *cis*- and *trans*-acting elements reponsible for the observed regulation of CD163 expression, we have cloned the promoter region of the CD163 gene [5]. This sequence contains binding sites for the transcription factors Sp1, C/EBPα, Ets-2, PU.1 and AP-1, which have been shown to play an important role in myeloid-specific gene expression. The promoter sequence also contains several binding sites for the glucocorticoid receptor probably mediating the strong induction of CD163 by dexamethasone. IL-10, a cytokine using Jak/STAT signalling pathways, also increases CD163 mRNA level. There are several consensus binding sites for STAT1 in the sequence, which might be important for the strong induction of CD163 mRNA by IL-10. In contrast to these anti-inflammatory stimuli, proinflammatory molecules decrease CD163 mRNA levels. LPS, the most

potent inflammatory stimulus, has been shown to mediate some of its actions by members of the Rel family. Although there are no consensus binding sites for Rel proteins located in the proximal promoter sequence, CD163 mRNA is significantly decreased after LPS stimulation of monocytes or macrophages. Since LPS has no effect on CD163 mRNA stability (data not shown), we assume that additional regulatory sequences or factors are involved in the downregulation of CD163 by LPS. The influence of the L1-transposable element, which was identified 1.4 kb upstream of the transcription start site, on the promoter activity has to be investigated.

Recently, several variants of CD163 have been described, which display altered extracellular domains or cytoplasmic tails. Regulated expression of these isoforms could provide another mechanism of controlling CD163 function. Interestingly, the cytoplasmic isoforms differ in their putative phosphorylation sites, suggesting that signalling through CD163 could be modulated by the use of different variants. Knowledge of the gene structure demonstrates that these isoforms of CD163 are generated by alternative splicing. Since the mRNAs encoding the cytoplasmic isoforms are found at significant levels in monocytes and macrophages, it may be suggested that these variants are not rare aberrant splicing products, but involve some kind of splice site regulation. Further functional and regulatory characterization of CD163 may help to understand the complete function of this protein.

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3 Kodelja V, Müller C, Tenorio S, Schebesch C, Orfanos CE, Goerdt S: Differences in angiogenic potential of classically vs alternatively activated macrophages. Immunobiology 1997; 197:478–493.

4 Buechler C, Ritter M, Orsó E, Langmann T, Klucken J, Schmitz G: Regulation of scavenger receptor CD163 expression in human monocytes and macrophages by pro- and antiinflammatory stimuli. In press.

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STIC-ILL

NPL

From: Jamroz, Margaret
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TI New human leukocyte clusters of differentiation
AU Stockinger, H.; Majdic, O.; Knapp, W.
SO Transfusion (Bethesda, Md.) (1998), 38(5), 499-505

TI Shedding of CD163, a novel regulatory mechanism for a member of the scavenger receptor cysteine-rich family.
AU Droste, Anne; Sorg, Clemens; Hoegger, Petra (1)
SO Biochemical and Biophysical Research Communications, (March 5, 1999) Vol. 256, No. 1, pp. 110-113.

TI Identification of the integral membrane protein RM3/1 on human monocytes as a glucocorticoid-inducible member of the scavenger receptor cysteine-rich family (CD163).
AU Hogger P; Dreier J; Droste A; Buck F; Sorg C
SO JOURNAL OF IMMUNOLOGY, (1998 Aug 15) 161 (4) 1883-90.

AU Ritter M; Buechler C; Langmann T; Orso E; Klucken J; Schmitz G
SO PATHOBIOLOGY, (1999) 67 (5-6) 257-61.

TI Regulation of scavenger receptor CD163 expression in human monocytes and macrophages by pro- and antiinflammatory stimuli.
AU Buechler, Christa; Ritter, Mirko; Orso, Evelyn; Langmann, Thomas; Klucken, Jochen; Schmitz, Gerd (1)
SO Journal of Leukocyte Biology, (Jan., 2000) Vol. 67, No. 1, pp. 97-103.

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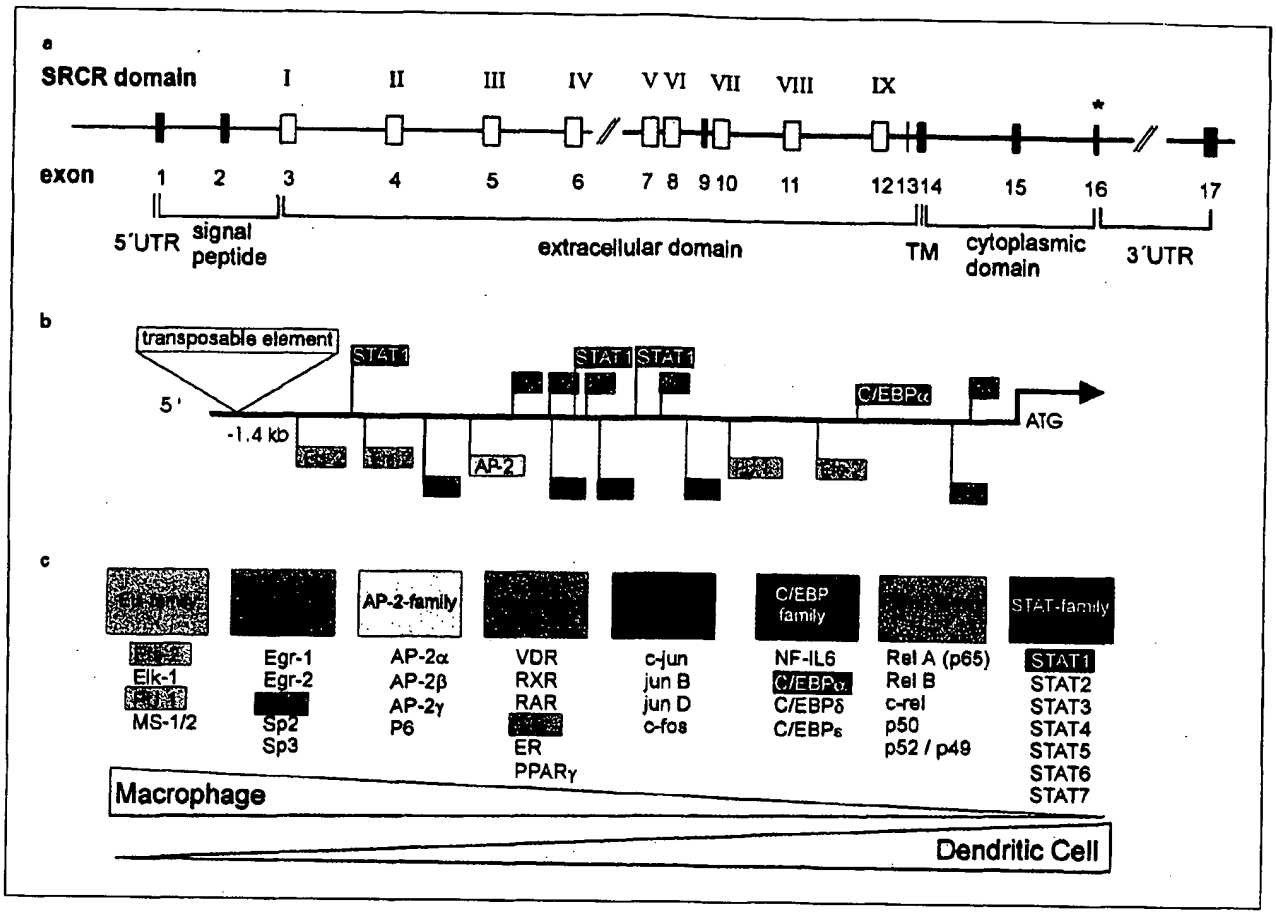

Fig. 1. a Genomic structure of the CD163 gene. Exons are denoted as boxes and gaps in the contiguous sequence are marked as double backslashes. Exons encoding SRCR domains are shown as yellow boxes. **b** Schematic drawing of the promoter region. Putative transcription factor binding sites are shown in boxes. The insertion of the L1-transposable element is indicated. **c** Transcription factors involved in phagocytic versus dendritic gene expression.

results in a suppression of CD163 gene transcription and in a decrease of the cell surface expression of CD163 protein. We also investigated the influence of the most potent inflammatory stimulus, lipopolysaccharide (LPS), on CD163 expression [4]. We found that in the presence of serum 1–10 ng/ml LPS are sufficient to induce a significant downregulation of CD163 mRNA in monocytes and macrophages. The same effect was observed, when monocytes or macrophages were stimulated with LPS in the absence of serum. However, under these conditions, much higher LPS concentrations (1 μg/ml) were necessary to induce a similar downregulation. The cell surface expression of CD163 on monocytes was also found to be downregulated following stimulation with LPS. This LPS-induced downregulation of CD163 may contribute to the absence of CD163-positive cells in early inflammation.

Promoter Structure of the CD163 Gene

In order to further characterize the transcriptional regulation of CD163 expression, we cloned the promoter region of the CD163 gene and determined the transcription initiation sites. Primer extension analysis identified one major transcription start site and six alternative initiation sites, which are used to a lesser extent. The adjacent 5′-flanking region contains several putative binding sites for transcription factors like PU.1, C/EBPα, Ets-2 and AP-1, which have been shown to play an important role in myeloid-specific gene expression (fig. 1b). There

are also potential glucocorticoid receptor binding sites, which might contribute to the strong induction of CD163 transcription following glucocorticoid treatment of monocytes and macrophages. The proximal sequence contains no TATA box, which is a common feature of many myeloid-specific promoter sequences and is in accordance with the observation of multiple transcription initiation sites. We also identified an L1-transposable element located 1.4 kb upstream of the major start site. Currently, we characterize the regulation of the promoter activity by pro- and anti-inflammatory stimuli and the influence of the L1 element.

Genomic Organization, Chromosomal Localization and Splice Variants of CD163

Regarding the number and spacing of cysteine residues per SRCR domain, the SRCR superfamily can be divided into two subfamilies and it has been proposed that these subfamilies differ in their genomic organization. The CD163 gene is composed of 17 exons and 16 introns and spans at least 35 kb (fig. 1a). The extracelluar portion of the mature protein is encoded by exons 1 through 14, with each SRCR domain being encoded by a separate exon. This exon organization is similar to other members of group B subfamily of the SRCR superfamily. While the SRCR domains of group B SRCR proteins seem to be encoded by single exons, SRCR domains of subfamily A proteins seem to be encoded by two exons. This indicates an independent evolution of group A and group B subfamilies.

Using fluorescent in situ hybridization with a fragment of the CD163 gene, which spanned the first three exons and introns, we mapped the CD163 gene to chromosome 12p13 [5]. Rearrangements of the short arm of chromosome 12 are one of the most frequently recurring motifs in a broad range of hematologic malignancies. Whether the CD163 gene is affected by these rearrangements has to be investigated.

Recently, several different forms of the CD163 mRNA have been described [1], which encode CD163 isoforms with altered cytoplasmic or extracellular domains. Information of the exon-intron boundaries of the gene reveals that all of these variants result from alternative splicing. Both described cytoplasmic isoforms arise from alternative splicing of intron 15. Since both variants are found at significant levels, it may be suggested that some kind of splice site regulation is involved in this splicing pattern. Another variant results from the retention of intron 7. The corresponding protein dislays an insertion of 33 aa between SRCR domain five and six. A putative truncated CD163 isoform, which is composed of only three SRCR domains and contains no transmembrane region, arises from alternative splicing of intron 5, which results in a frame shift and a premature termination of translation. However, the functional differences of these isoforms have to be evaluated.

Discussion

CD163 is a member of the SRCR superfamily that is exclusively expressed on monocytic cells. CD163 is abundant on most tissue macrophages, while dendritic cells are not stained with CD163-specific mAbs. We demonstrate that in vitro phagocytic differentiation of monocytes induces CD163 mRNA and protein expression, while dendritic differentiation of these cells suppresses the expression of CD163. Because CD163 expression is associated with the anti-inflammatory response of monocytes and macrophages, we were also interested in the regulation of CD163 by pro- and anti-inflammatory mediators. Our results show that proinflammatory stimuli including the cytokines IFN-γ and TNF-α as well as LPS suppress CD163 mRNA and protein in monocytes and macrophages [4]. The abundance of these stimuli in early inflammation may contribute to the absence of CD163-positive cells in this phase. In contrast, the anti-inflammatory cytokine IL-10 and the glucocorticoid dexamethasone both induce a rapid upregulation of CD163 in monocytes and macrophages. A similar effect was observed for IL-6. Since IL-10 and IL-6 secretion are induced by IFN-γ and LPS, we assume that these cytokines mediate the high expression of CD163 on anti-inflammatory macrophage subsets.

In order to identify *cis*- and *trans*-acting elements reponsible for the observed regulation of CD163 expression, we have cloned the promoter region of the CD163 gene [5]. This sequence contains binding sites for the transcription factors Sp1, C/EBPα, Ets-2, PU.1 and AP-1, which have been shown to play an important role in myeloid-specific gene expression. The promoter sequence also contains several binding sites for the glucocorticoid receptor probably mediating the strong induction of CD163 by dexamethasone. IL-10, a cytokine using Jak/STAT signalling pathways, also increases CD163 mRNA level. There are several consensus binding sites for STAT1 in the sequence, which might be important for the strong induction of CD163 mRNA by IL-10. In contrast to these anti-inflammatory stimuli, proinflammatory molecules decrease CD163 mRNA levels. LPS, the most

potent inflammatory stimulus, has been shown to mediate some of its actions by members of the Rel family. Although there are no consensus binding sites for Rel proteins located in the proximal promoter sequence, CD163 mRNA is significantly decreased after LPS stimulation of monocytes or macrophages. Since LPS has no effect on CD163 mRNA stability (data not shown), we assume that additional regulatory sequences or factors are involved in the downregulation of CD163 by LPS. The influence of the L1-transposable element, which was identified 1.4 kb upstream of the transcription start site, on the promoter activity has to be investigated.

Recently, several variants of CD163 have been described, which display altered extracellular domains or cytoplasmic tails. Regulated expression of these isoforms could provide another mechanism of controlling CD163 function. Interestingly, the cytoplasmic isoforms differ in their putative phosphorylation sites, suggesting that signalling through CD163 could be modulated by the use of different variants. Knowledge of the gene structure demonstrates that these isoforms of CD163 are generated by alternative splicing. Since the mRNAs encoding the cytoplasmic isoforms are found at significant levels in monocytes and macrophages, it may be suggested that these variants are not rare aberrant splicing products, but involve some kind of splice site regulation. Further functional and regulatory characterization of CD163 may help to understand the complete function of this protein.

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STIC-ILL

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TI New human leukocyte clusters of differentiation
AU Stockinger, H.; Majdic, O.; Knapp, W.
SO Transfusion (Bethesda, Md.) (1998), 38(5), 499-505

TI Shedding of CD163, a novel regulatory mechanism for a member of the scavenger receptor cysteine-rich family.
AU Droste, Anne; Sorg, Clemens; Hoegger, Petra (1)
SO Biochemical and Biophysical Research Communications, (March 5, 1999) Vol. 256, No. 1, pp. 110-113.

TI Identification of the integral membrane protein RM3/1 on human monocytes as a glucocorticoid-inducible member of the scavenger receptor cysteine-rich family (CD163).
AU Hogger P; Dreier J; Droste A; Buck F; Sorg C
SO JOURNAL OF IMMUNOLOGY, (1998 Aug 15) 161 (4) 1883-90.

AU Ritter M; Buechler C; Langmann T; Orso E; Klucken J; Schmitz G
SO PATHOBIOLOGY, (1999) 67 (5-6) 257-61.

TI Regulation of scavenger receptor CD163 expression in human monocytes and macrophages by pro- and antiinflammatory stimuli.
AU Buechler, Christa; Ritter, Mirko; Orso, Evelyn; Langmann, Thomas; Klucken, Jochen; Schmitz, Gerd (1)
SO Journal of Leukocyte Biology, (Jan., 2000) Vol. 67, No. 1, pp. 97-103.

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Regulation of scavenger receptor CD163 expression in human monocytes and macrophages by pro- and antiinflammatory stimuli

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Abstract: CD163, also referred to as M130, a member of the scavenger receptor cysteine-rich family (SRCR) is exclusively expressed on cells of the monocyte lineage. In freshly isolated monocytes the $CD14^{bright}$ $CD16^{+}$ monocyte subset revealed the highest expression of CD163 among all monocyte subsets. CD163 mRNA and protein expression is up-regulated during macrophage colony-stimulating factor (M-CSF)-dependent phagocytic differentiation of human blood monocytes. In contrast, monocytic cells treated with GM-CSF and interleukin-4 (IL-4) for dendritic differentiation down-regulate this antigen. CD163 expression is also suppressed by proinflammatory mediators like lipopolysaccharide (LPS), interferon-γ (IFN-γ), and tumor necrosis factor α, whereas IL-6 and the antiinflammatory cytokine interleukin-10 (IL-10) strongly up-regulate CD163 mRNA in monocytes and macrophages. The effects of the immunosuppressants dexamethasone, cyclosporin A (CA), and cortisol differ in their capacity to influence CD163 mRNA levels. Our results demonstrate that CD163 expression in monocytes/macrophages is regulated by proinflammatory and antiinflammatory mediators. This expression pattern implies a functional role of CD163 in the antiinflammatory response of monocytes. *J. Leukoc. Biol.* 67: 97–103; 2000.

Key Words: *interleukin-10 · interferon-γ · inflammation · tumor necrosis factor α*

INTRODUCTION

The protein M130, recently classified as CD163, has been characterized as a member of the scavenger receptor cysteine-rich (SRCR) superfamily. Its expression seems to be restricted to the monocyte/macrophage lineage [1, 2].

The CD163 protein was recently purified and the cloning of the corresponding cDNA revealed multiple splice variants of this gene. The predominant mRNA form contains an open reading frame of 3715 bp encoding a transmembrane segment of 24 amino acids, a short cytoplasmic region of 49 amino acids, and 9 SRCR domains [3]. Comparative structural analysis revealed the SRCR domain as a protein motif in different proteins, including the WC1 antigen, CD5, CD6, and Spα [reviewed in 4, 5]. Several of these proteins are expressed in leukocytes and have been implicated in the development and regulation of the immune system. Among these proteins, CD163 is most homologous to the WC1 antigen, which is exclusively expressed on γδ T cells [6]. These T cells are regarded as part of the innate immunity, the nonadaptive, first line of host defense [7].

Immunofluorescent and immunocytochemical analysis with the monoclonal antibodies Ki-M8, Ber-MAC3, GHI/61, and SM4 against different epitopes of CD163 demonstrated expression of M130 on human blood monocytes and in most tissue macrophages [1, 8]. CD163 is expressed on the cell surface and in the intracellular compartment as well [1–3] and it was suggested that a soluble form of CD163 may be secreted from the cells [2]. Dendritic cells like Langerhans cells do not express the CD163 protein [2].

More recently Högger et al. [9] demonstrated that the monoclonal antibody RM3/1 also recognizes CD163. RM3/1-positive cells develop *in vivo* during the healing phase of acute inflammatory reactions [10, 11] and were shown to produce antiinflammatory and angiogenic factors [12, 13]. On the other hand, interferon-γ (IFN-γ)-induced macrophages do not express CD163 protein [14]. They secrete a variety of proinflammatory cytokines and are present in early inflammation [10, 14]. From these data it was suggested that CD163 expression is associated with antiinflammatory processes [10, 15] and that RM3/1 stained monocytes are involved in the down-regulation of the inflammatory response [11].

The RM3/1 antibody was shown to inhibit the adhesion of dexamethasone-treated monocytes to activated endothelial cells, indicating a function of CD163 in the adhesion of monocytes to activated endothelium [16]. These authors suggest that in the antiinflammatory phase CD163-expressing monocytes are recruited from the blood and adhere to the endothelium, whereas in early inflammation CD163-negative monocytes adhere via different adhesion proteins [16].

To further characterize the regulation and function of CD163 we examined the effects of pro- and antiinflammatory stimuli on its expression in monocytes and macrophages. CD163 mRNA as well as protein expression is down-regulated on monocyte

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activation with lipopolysaccharide (LPS), IFN-γ, and tumor necrosis factor α (TNF-α). However, IL-6 and the antiinflammatory cytokine IL-10 strongly induce CD163 mRNA in mononuclear cells. The immunosuppressant cortisol and cyclosporin A do not alter CD163 mRNA expression significantly, whereas dexamethasone strongly induces CD163 mRNA. Our results indicate that CD163 expression is substantially regulated by mediators of the inflammatory response and is strongly induced in macrophages by antiinflammatory stimuli.

MATERIALS AND METHODS

Reagents

Human recombinant macrophage colony-stimulating factor (M-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-4 were obtained from Genzyme Diagnostics (Cambridge, MA). The monoclonal antibody Ki-M8 was ordered from Biomedicals AG (Augst). RPMI and macrophage SFM medium was purchased from GIBCO-BRL (Karlsruhe). Other laboratory reagents, cytokines, and chemicals were purchased from Sigma Chemical (Deisenhofen) unless noted otherwise. IL-10 was ordered from R & D Systems (Wiesbaden-Nordenstadt). Membranes (Genescreen) for Northern blotting were ordered from NEN Life Sciences (Boston) and [α-^{32}P]dCTP from Amersham (Braunschweig).

Isolation and culture of cells

Peripheral blood monocytes from healthy volunteers were isolated by leukapheresis followed by elutriation according to standard protocol [17] or by density gradient centrifugation over Histopaque-1077 [18]. Fractions containing >90% monocyte purity were pooled and cultured on plastic Petri dishes (10^6 cells/mL) in a serum-free macrophage SFM medium supplemented with the indicated stimuli.

For flow cytometric analysis, the monocytes were seeded in 20-cm^2 PetriPERM dishes with a hydrophobic Teflon bottom at a concentration of 10^6 cells/mL.

All cell lines used were obtained from ATCC with the exception of SU-DHL-1 cells, which were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig). Cells were cultured in RPMI-1640 medium (Sigma) supplemented with 10% fetal calf serum (GIBCO-BRL), and incubated in 10% CO_2 in air at 37°C. To induce phagocytic differentiation cells were cultured in the presence of 160 nM phorbol myristate acetate (PMA) for 48 h.

Recombinant human cytokines and biological active substances

The following cytokines were used for mononuclear cell cultures: GM-CSF, 5 ng/mL in combination with IL-4, 10 ng/mL; M-CSF, 5 ng/mL; IL-10, 10 ng/mL; IL-6, 10 ng/mL; IFN-γ, 10 ng/mL; and TNF-α, 10 ng/mL. Additional bioactive substances applied were dexamethasone, 40 ng/mL; cortisol, 4 ng/mL; cyclosporin A, 0.1 μg/mL, and LPS, *Escherichia coli* serotype 055:B5, 1, 10, or 100 ng/mL in the presence and 1 μg/mL in the absence of serum.

Isolation of RNA and Northern blot analysis

Total cellular RNA was isolated by the guanidine isothiocyanate-cesium chloride technique [19]. Ten micrograms of total RNA was separated through a 1.2% agarose gel containing 6% formaldehyde and blotted onto nylon membranes. After cross-linking with ultraviolet (UV) irradiation (Stratalinker model 1800, Stratagene, La Jolla, CA), the membranes were hybridized with a cDNA probe spanning nucleotides 2959 to 3424 of the CD163 cDNA [3], stripped, and subsequently hybridized with a human GAPDH or β-actin probe (Clontech, Palo Alto, CA). The probes were radiolabeled with [α-^{32}P]dCTP using the Oligolabeling kit from Pharmacia (Freiburg). Hybridization and washing conditions were performed as recommended by the manufacturer of the membrane.

Competitive polymerase chain reaction (PCR)

To analyze CD163 mRNA expression a competitive PCR was established [20]. The competitor ranging from nucleotide 3226 to 3551 [3] was amplified by RT-PCR with the primers Puni (5'CCATAGTGAGTGTGGGCACAA3') and Prev (5'TCAGTGTGGCTCA GAATGGCCTC 3') and cloned into the plasmid pJOE533 kindly provided by Dr. Altenbuchner. *Eco*RI digestion and subsequent ligation resulted in the deletion of an internal 39-bp fragment.

Total RNA (1.2 μg) was reverse transcribed and PCR was performed with the primer Puni and Prev (RT-PCR Kit, Perkin-Elmer). The competitor (0.12 μg/μL) was added to the PCR reaction in different concentrations. The PCR products were analyzed on 3% agarose gels. PCR with an equivalent concentration of cDNA or the competitor DNA results in two DNA fragments that differ in size by 39 bp. An excess of either the cDNA or competitor produces just one fragment.

Staining for immunofluorescence and flow cytometric analysis of cultured monocytes

To obtain cell suspensions from cultured monocytes the cells were harvested by gentle scratching with a cell scraper after a 30-min incubation on ice. The cell number was adjusted to 5×10^5 cells after resuspension in Dulbecco's modified phosphate-buffered saline without Ca^{2+} and Mg^{2+} (DPBS). The cells were then stained for 15 min on ice with saturating concentrations of the following fluorochrome-conjugated antibodies: anti-CD16a (clone 3G8) as a fluorescein isothiocyanate (FITC) conjugate from Coulter-Immunotech (Hamburg, Germany), anti-CD163 (clone GHI/61) as an R-phycoerythrin (R-PE) conjugate from PharMingen (Hamburg, Germany), and anti-CD14 (clone MφP9) as allophycocyanin (APC) conjugate from Becton Dickinson (Heidelberg, Germany). After subsequent washing steps the fluorescence signals were detected by a FACSCalibur flow cytometer (Becton Dickinson). The data were analyzed and expressed as mean fluorescence intensity by using the Attractors software package (Becton Dickinson). The cells were not permeabilized and only the surface expression of CD163 was analyzed.

The determination of the monocyte subsets was performed as described previously [21]. Data are presented as expression density in percentage of unstimulated monocytes and/or of monocyte subset 1 as mean ± SEM of the number of independent experiments.

RESULTS

Expression of CD163 in cultured monocytes, phagocytes, and dendritic cells

It has long been known that circulating monocytes represent a heterogeneous cell population that can be divided into at least four subpopulations with regard to their expression of the LPS receptor CD14 and the FcγRIIIa receptor CD16a [21, 22]. In freshly isolated and up to 3-day cultured human monocytes the four subsets (MNP1-MNP4) are clearly distinguished due to the level of CD14 and CD16 expression (**Fig. 1A**). Regarding the expression of CD163 in monocyte subsets we found that CD14^{bright}CD16a$^+$ cells referred to as MNP2 in Figure 1A with a high phagocytic capacity [21, 22] revealed the highest antigen density for CD163 (Fig. 1B). This result is in accordance with previous data that MNP2 cells represent a more mature, probably prephagocytic, phenotype of monocytes. However, the pool size of the monocyte subsets are highly variable when different donors are compared, and is influenced by genetic factors like the ApoE phenotype [18]. This may be the reason for the wide range described for the expression of CD163 in blood monocytes ranging from 5 [2] to 30% [9] of freshly isolated monocytes. We would like to emphasize that the expression of CD163 in the different subsets was determined

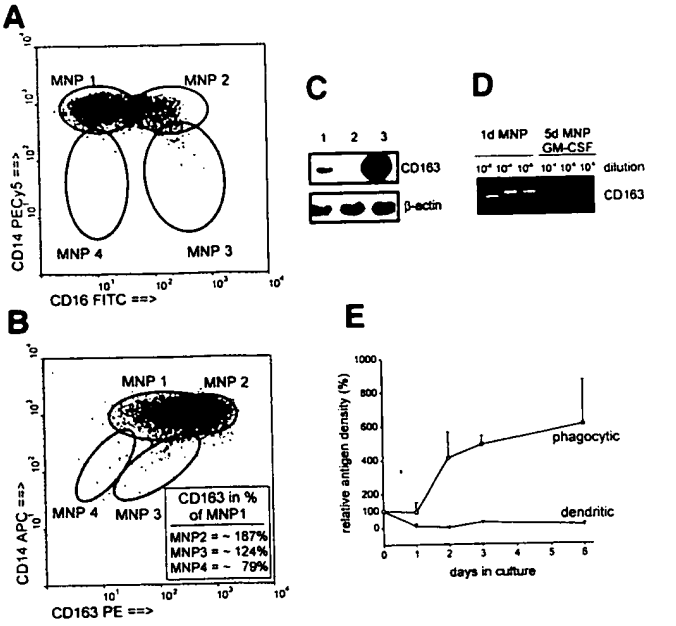

Fig. 1. (A) Characterization of mononuclear phagocyte subsets in 1-day-cultured monocytes on their coexpression patterns of CD14 and CD16a. Approximately 60% of monocytes belong to the subset 1 (MNP1) that is characterized by bright expression of CD14 and no or dim expression of CD16a. The second (MNP2) and third (MNP3) subsets have high capacity for phagocytosis and have bright expression of CD16a and bright (MNP2) or dim (MNP3) expression of CD14, respectively. The fourth subset (MNP4) is known to be a precursor subpopulation for antigen-presenting (dendritic) cells as they present only dim expression of CD14 and CD16a. (B) Surface expression of CD163 (CD163) in the four monocyte subsets. The antigen density of CD163 in MNP1 was calculated as 100% and the expression of CD163 in the other three subsets was expressed as percent of MNP1. (C) Northern blot analysis of CD163 expression with RNA isolated from monocytes (lane 1), *in vitro*-differentiated dendritic cells (lane 2, 5-day GM-CSF, IL-4) and *in vitro*-differentiated phagocytes (lane 3, 5-day M-CSF). The blot was stripped and reprobed for β-actin expression. (D) Competitive PCR with RNA isolated from monocytes and monocytes incubated for 5 days with GM-CSF and IL-4. The dilution of the competitor plasmid (0.12 μg/mL) is indicated above. (E) Surface expression of CD163 in cultured human monocytes on *in vitro* phagocytic and dendritic differentiation, as assessed by flow cytometry. The data are expressed as relative antigen density in percent of the expression of freshly isolated cells.

after incubating the cells for 24 h with M-CSF. Our data provide information about the CD163 antigen density on the monocyte subsets and not about the abundance of $CD163^+$ cells in whole blood.

Considering that a reliable quantification of monocyte subpopulations on *in vitro* culture is only possible for up to 3 days [18], the monitoring of the expression of CD163 during *in vitro* phagocytic or dendritic differentiation was performed in the total cell population.

The expression of CD163 mRNA in *in vitro*-differentiated phagocytic and dendritic cells was examined by Northern blot analysis (Fig. 1C). CD163 mRNA was strongly up-regulated (lane 3) when monocytes were cultured in the presence of M-CSF. Dendritic differentiation induced by GM-CSF and IL-4 suppressed CD163 mRNA expression (lane 2). Using competitive RT-PCR we demonstrate a 10-fold reduction of CD163 mRNA in dendritic cells when compared with monocytes (Fig. 1D). A subsequent flow cytometric analysis confirmed that the surface expression of CD163 strongly increases during *in vitro* phagocytic differentiation (up to sixfold), whereas it declines and is hardly detectable during *in vitro* dendritic differentiation (Fig. 1E).

LPS suppresses CD163 mRNA and protein expression

Surface expression of CD163 was found to be associated with the release of antiinflammatory and angiogenic products from macrophages [11, 13]. Expression of the CD163 antigen occurs in late inflammation and is associated with the down-regulation of the inflammatory response and angiogenesis during inflammation. We were interested in the regulation of CD163 mRNA and protein expression by the most potent proinflammatory mediator, LPS, that was shown to mimic many of the responses of monocytes/macrophages against invading bacteria [23]. LPS at a concentration of 1 ng/mL is enough to activate monocytes in serum due to the transfer activity of lipopolysaccharide-binding protein (LBP). In serum-free media much higher doses of LPS are required to achieve identical responses [24].

When monocytes were differentiated in the presence of 10% fetal calf serum (FCS) for 8, 25, or 32 h even 1 ng/mL LPS suppressed CD163 mRNA expression (**Fig. 2A**). Higher LPS

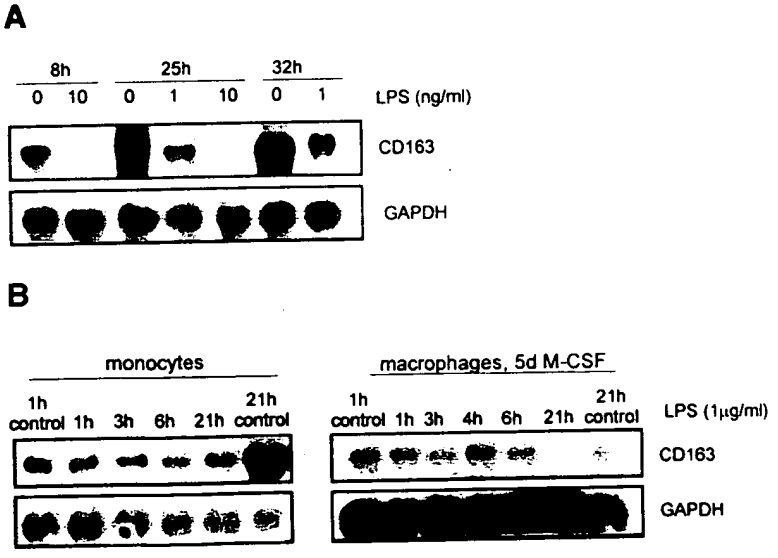

Fig. 2 (A) LPS-mediated suppression of CD163 mRNA in monocytes cultured in the presence of serum and different concentrations of LPS for 8, 25, and 32 h. RNA was prepared and used to determine CD163 and GAPDH mRNA by Northern blot analysis. (B) LPS-mediated suppression of CD163 mRNA in 1- and 5-day M-CSF-differentiated macrophages. Cells were incubated with 1 μg/mL LPS in serum-free medium for the indicated time. Total RNA was isolated and analyzed by Northern blot for CD163 expression. The blot was stripped and reprobed with a GAPDH probe.

doses further down-regulate CD163 mRNA expression. Also, when monocytes were cultured for 1 or 6 days in medium supplemented with M-CSF, LPS (1 μg/mL) induced a significant down-regulation of CD163 mRNA expression within 21 h in 1-day MNP and 5-day MNP, respectively (Fig. 2B). The surface expression of CD163 protein was analyzed by flow cytometry in monocytes isolated from seven different healthy donors and cultured with LPS for 24 h. LPS-treated cells reduced their CD163 surface expression by 46.4 ± 7.1% compared with control cells (see Fig. 4).

The down-regulation of CD163 by LPS may at least partly contribute to the absence of CD163-positive cells in the early phase of inflammation.

The proinflammatory cytokines IFN-γ and TNF-α suppress CD163 expression

Complete activation of macrophages during inflammatory responses can be achieved by stimulation with LPS and IFN-γ [23]. One-day MNP were incubated with 10 ng/mL of IFN-γ for 18 h, and RNA expression was determined by Northern blot analysis. As expected from the LPS data, IFN-γ suppressed CD163 mRNA expression in monocytes (**Fig. 3A**, lane 3). The identical result was obtained by competitive PCR (not shown). CD163 mRNA is 10-fold less in these cells compared with control monocytes. The surface expression of CD163 was reduced by IFN-γ to 17.9 ± 11.6% (**Fig. 4**) within 24 h. The effects of IFN-γ were also investigated in macrophages. Monocytes were cultured with M-CSF for 5 days. IFN-γ was added and CD163 mRNA expression was analyzed after 8 and 24 h incubation with the cytokine. Whereas within the first 8 h

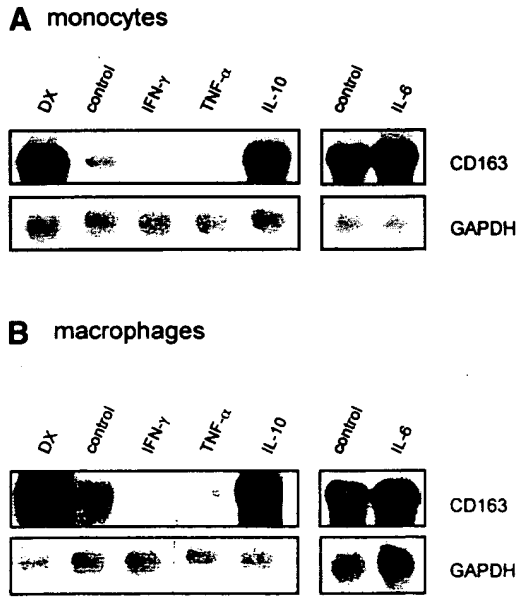

Fig. 3. (A) Effects of IFN-γ, TNF-α, IL-10, IL-6, and DX on CD163 expression were analyzed. Monocytes were incubated for 18 h with different stimuli or in medium alone (control). RNA was isolated and used to determine CD163 mRNA expression. The blots were stripped and reprobed with GAPDH to confirm equal RNA loading. (B) Effects of IFN-γ, TNF-α, IL-10, IL-6, and DX on CD163 expression were analyzed. Monocytes differentiated to macrophages with M-CSF (5 days) were incubated with the indicated stimuli or in medium alone (control) for 18 h. RNA was prepared and a Northern blot was performed to determine CD163 and GAPDH mRNA expression.

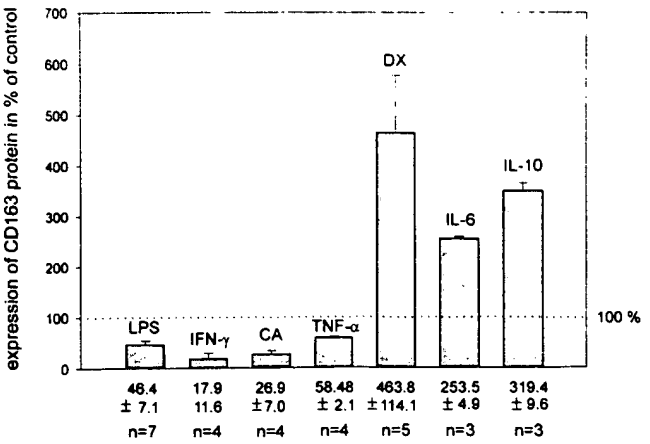

Fig. 4. Monocytes were incubated with the indicated stimuli for 24 h and CD163 expression was determined by flow cytometric analysis. CD163 surface expression is given in percentage of unstimulated monocytes. *n*, Number of independent experiments using monocytes from different probands.

CD163 mRNA levels are comparable to the control cells (data not shown), incubation with IFN-γ for 18 h significantly reduced CD163 mRNA in macrophages (Fig. 3B, lane 3).

The proinflammatory cytokine TNF-α (10 ng/mL), which is detectable early in the supernatants of monocytes activated by LPS, also suppresses CD163 mRNA in monocytes (Fig. 3A, lane 4) and macrophages within 18 h (Fig. 3B, lane 4). In monocytes, incubation with TNF-α for 24 h resulted in a reduced surface expression of CD163 by 58.48 ± 2.1% (Fig. 4). These experiments indicate that CD163 expression is substantially suppressed by the proinflammatory cytokines TNF-α and IFN-γ and may explain the absence of CD163-positive cells in early inflammation.

IL-6 and IL-10 exert similar effects on CD163 expression

Compared to LPS and IFN-γ, antiinflammatory cytokines such as IL-10 exert a wide range of antagonistic effects on monocytes/macrophages [25, 26]. IL-10 is induced by TNF-α and down-regulates the expression of TNF-α [27]. We first examined the influence of IL-10 on CD163 mRNA expression. IL-10 significantly induced CD163 mRNA in monocytes (Fig. 3A, lane 5) and macrophages (Fig. 3B, lane 5) within 18 h. Up-regulation of CD163 mRNA was observed within 2 h (not shown) in monocytes and macrophages. In parallel, monocytes incubated in the presence of IL-10 up-regulate CD163 surface expression by a factor of three (Fig. 4).

We also investigated the influence of IL-6 on CD163 mRNA and protein levels and found that although mRNA expression is not significantly changed by IL-6 incubation (Fig. 3, A and B, lane 7), CD163 surface expression is induced by 253.5 ± 4.9% (Fig. 4). IL-6 is a multifunctional cytokine [28] and is described to exert proinflammatory [29] and antiinflammatory effects on monocytes [30]. It is also known to induce macrophage maturation [28]. IL-10 and IL-6 are induced by TNF-α [27, 28] and these cytokines may mediate the up-regulation of CD163 in the antiinflammatory response.

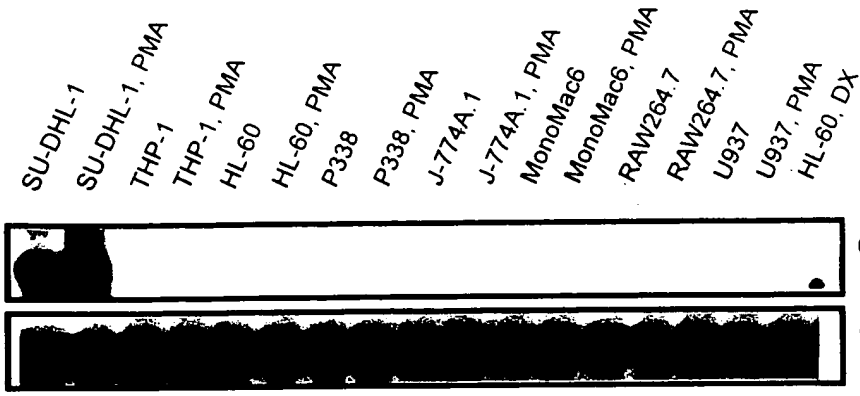

Fig. 5. CD163 expression in different monocytic cell lines was investigated. Cells were incubated in medium alone, with PMA for 48 h or dexamethasone for 24 h. RNA was isolated and CD163 and GAPDH expression was determined by Northern blot.

Influence of immunosuppressants on CD163 mRNA

Cyclosporin A, a drug used to suppress immunoresponses in several inflammatory diseases, did not influence gene expression either in 1-day MNPs (data not shown) or in 6-day MNPs. However, FACS analysis revealed that cyclosporin A-treated monocytes have a reduced CD163 surface expression (Fig. 4), as was shown earlier by Wenzel et al. [16]. The mechanisms of this down-regulation need to be evaluated by further experiments. We confirmed previous data that dexamethasone strongly induced CD163 mRNA (Fig. 3A, lane 1) and protein expression (Fig. 4) in 1-day MNP [9]. CD163 mRNA is also induced in differentiated macrophages (Fig. 3B, lane 1) within 18 h. The effect is seen even in cells incubated just for 2 h (not shown). Cortisol did not alter CD163 mRNA levels in monocytes and macrophages (not shown). These experiments demonstrate that immunosuppressants induce different functional changes on monocytes/macrophages.

Expression of CD163 in monocytic cell lines

The expression of CD163 mRNA was investigated in different human and mouse cell lines of monocytic origin in order to have a suitable *in vitro* model for further investigations. These cell lines differentiate along the monocytic lineage after exposure to PMA [31]. The cell lines investigated were as follows: THP-1 [32], U937 [33], HL-60 [34], P338 [35], MonoMac6 [36], RAW264.7 [37], and J-774A.1 [38]. None of these cell lines express CD163 mRNA to a level detectable by Northern blot analysis even when the cells are stimulated with PMA (**Fig. 5**). To increase the sensitivity of mRNA detection, CD163 mRNA expression of HL-60 cells was investigated by competitive PCR analysis. We found that the amount of mRNA detectable in undifferentiated HL-60 cells is ninefold lower compared with human blood monocytes. However, incubation with dexamethasone for 24 h increased CD163 mRNA expression in HL-60 cells to a level detectable by Northern blot analysis (Fig. 5).

The only cell line with high expression of CD163 is the true histiocytic lymphoma cell line SU-DHL-1, described as monocytic M5-type cells [3].

Influence of pro- and antiinflammatory cytokines on CD163 expression in SU-DHL-1

M-CSF-dependent phagocytic differentiation strongly induces CD163 expression in monocytes/macrophages (Fig. 1C). PMA was used to induce phagocytic differentiation in the SU-DHL-1 cell line. When SU-DHL-1 cells were treated with PMA, the cells became adherent, stopped proliferating, and developed a phagocytic phenotype. CD163 mRNA (Fig. 5 and **Fig. 6**) and protein expression (not shown) was up-regulated by incubating the SU-DHL-1 cells with PMA for 24 h (Figs. 5 and 6). As was shown above for monocytes/macrophages, LPS also suppressed CD163 expression in these cells (Fig. 6).

Although DX alone did not induce CD163 expression, the combination of DX and PMA slightly increased CD163 expression over PMA alone (Fig. 6). The SU-DHL-1 cell line may be used to investigate transcriptional regulation of the CD163 gene in the context of differentiation and LPS activation. But because this cell line is poorly characterized investigations concerning the expression of further monocytic surface antigens like CD14 and CD16 should be performed simultaneously.

DISCUSSION

CD163 is of special interest because its expression is restricted to monocytic cells [1]. The protein is present on peripheral monocytes and we found that the MNP2 subpopulation representing a more mature, phagocytic phenotype of monocytes [39]

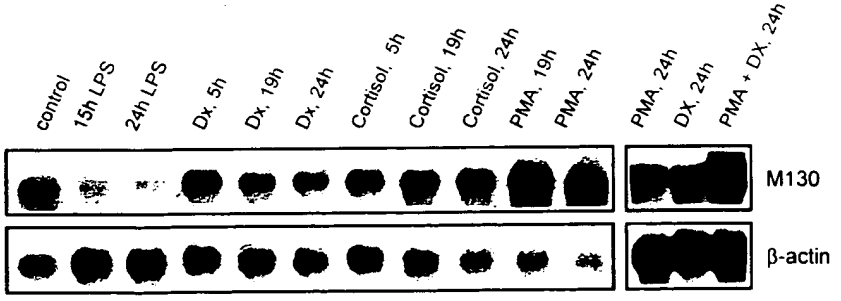

Fig. 6. CD163 expression in the SU-DHL-1 cell line was investigated. The cells were incubated with LPS, dexamethasone, cortisol, or PMA for the indicated time. RNA was prepared and CD163 and β-actin expression were analyzed by Northern blot.

has the highest density of the CD163 antigen among the four monocyte subsets distinguished by their expression of CD14 and CD16a. The MNP2 subpopulation displays expression of CD16a and high expression of CD14 and CD163. Whether the coexpression of CD14 and CD163 on the same monocyte subset has any functional importance has yet to be investigated.

CD163 is abundant on most tissue macrophages, however, on dendritic cells, CD163 protein was not detectable by staining with the mAb Ber-Mac3 [2]. Here we show that *in vitro* differentiation of monocytes to macrophages by M-CSF in serum-free medium strongly induces CD163 mRNA and protein expression. When the monocytes were differentiated to dendritic cells by GM-CSF and IL-4 [40] CD163 mRNA and protein levels were suppressed. The function of the CD163 protein is still unknown. However, recent data demonstrating that the monoclonal antibody RM3/1 (as well as the mAbs Ki-M8, SM4, BerMac3, and GHI/61), recognizes the CD163 protein [9], indicate a function of CD163 in inflammatory processes. The expression of the RM3/1 antigen is associated with an antiinflammatory macrophage subset [41] present in the down-regulatory phase of an immune response [10].

It was shown that the adherence of RM3/1-positive monocytes to endothelial cells is affected by cytokine treatment of the endothelium [42] and we were interested in whether the expression of CD163 is in addition regulated by pro- and antiinflammatory mediators present in early or late immune response. The results presented here show that the proinflammatory cytokines IFN-γ and TNF-α as well as LPS significantly suppress CD163 mRNA and protein expression. Other groups describe no influence of LPS on CD163 expression. However, in these studies higher LPS concentrations (1 and 10 μg/mL in the presence of serum) [2, 43] were used. Therefore, the results are difficult to compare.

This is the first work to investigate the effects of the proinflammatory mediators LPS, IFN-γ, and TNF-α on the mRNA and protein levels of CD163. We demonstrate that CD163 expression is down-regulated by these stimuli to a different extent. The abundance of these cytokines and possibly LPS in early inflammation may contribute to the absence of CD163-positive cells described elsewhere [16].

We also investigated the influence of the antiinflammatory cytokine IL-10 on CD163 mRNA regulation. IL-10 is well documented as a negative regulator of LPS-induced gene expression in macrophages [25, 26, 44] and it is reasonable to assume an IL-10-mediated up-regulation of CD163 mRNA. Indeed, IL-10 significantly induced CD163 expression in monocytes and macrophages.

IL-6 also increased CD163 protein expression within 24 h. IL-6 is a multifunctional cytokine important for host defense and was also shown to induce phagocytic differentiation in monocytes [28]. Expression of IL-6 and IL-10 is induced by LPS and TNF-α [27, 28] and we speculate that these cytokines participate in the up-regulation of CD163 in late inflammation.

The abundance of CD163 protein was also studied in monocytic cell lines. We investigated the CD163 mRNA expression in human and mouse macrophage cell lines by Northern blot analysis and could not detect any CD163 mRNA even when phagocytic differentiation was induced by PMA. However, mRNA expression is up-regulated by dexamethasone, as was demonstrated for HL-60 cells in this work. The only cell line with high expression of CD163 protein and mRNA is the SU-DHL-1 cell line [our unpublished results and ref. 2]. However, the origin of these cells is unclear. Some authors describe the SU-DHL-1 cells from lymphoid origin [45], others refer to them as monocytic cells [46].

To have a suitable model for further investigations, CD163 mRNA expression was studied in the SU-DHL-1 cell line. PMA, which is known to initiate maturation in monocytic cell lines [31], induced adherence of these cells to plastic dishes. We found that PMA up-regulates CD163 mRNA and protein in SU-DHL-1 cells. Treatment with dexamethasone and PMA further induced CD163 mRNA abundance compared to PMA alone, whereas dexamethasone alone was not effective. The incubation with LPS resulted in a drastic suppression of CD163 mRNA. These results indicate that CD163 gene expression in SU-DHL-1 is regulated on differentiation and LPS incubation as it is seen in monocytes; dexamethasone, however, had no effect. Therefore the use of the SU-DHL-1 cells as a model for *in vitro* studies of CD163 regulation is limited.

It has been proposed that RM3/1-positive macrophages are involved in antiinflammatory responses [11]. Expression of this antigen was shown to be associated with the secretion of antiinflammatory [12, 47] and angiogenic [13] factors. Wenzel et al. [16] suggested that in early inflammation endothelial cells recruit RM3/1-negative, proinflammatory monocytes and later on CD163-positive, antiinflammatory cells. These cells adhere to the endothelium via CD163 expressed on monocytes and a so far unknown counterpart on the endothelium.

It was clearly shown that the RM3/1-positive monocytes preferentially adhere to IL-6-treated endothelium, whereas IFN-γ- and TNF-α-incubated endothelial cells recruit less RM3/1-positive monocytes [42]. In addition, IFN-γ and TNF-α suppress CD163 expression shown in this work. Later, when IL-6 and IL-10 are secreted, the expression of CD163 is rapidly induced. Therefore, we speculate that the expression of the RM3/1 antigen in early and late inflammation is regulated by various cytokines and does not only reflect the recruitment of a specific monocyte subpopulation from the blood compartment [43]. Taken together, CD163 seems to play an important function in phagocytes and in the inflammatory response of monocytes and macrophages to invading bacteria.

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Regulation of CD163 on human macrophages: cross-linking of CD163 induces signaling and activation

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Abstract: CD163 is a member of the group B scavenger receptor cysteine-rich (SRCR) superfamily. This study describes aspects of the tissue distribution, the regulation of expression, and signal transduction after cross-linking of this receptor at the cell surface of macrophages. CD163 showed an exclusive expression on resident macrophages (e.g., red pulp macrophages, alveolar macrophages). The expression was inducible on monocyte-derived macrophages by glucocorticoids but not by interleukin-4 (IL-4), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interferon-γ. The combination of IL-4 or GM-CSF with glucocorticoids resulted in a further increase. Subcellular analysis of alveolar macrophages by immunoelectron microscopy showed a plasma membrane localization of the antigen. Cross-linking of CD163 with monoclonal antibody induced a protein tyrosine kinase-dependent signal that resulted in (1) slow-type calcium mobilization, (2) inositol triphosphate production, and (3) secretion of IL-6 and GM-CSF. The data suggest a function for the SRCR-superfamily receptor CD163 in the regulation of inflammatory processes by macrophages. *J. Leukoc. Biol.* 66: 858–866; 1999.

Key Words: *innate immunity · glucocorticoid · human · resident macrophage*

INTRODUCTION

Macrophages play a central role in inflammatory processes [1]. Their function reaches from phagocytosis of microorganisms or particles up to the regulation of local immune response by the production of cytokines and other types of mediators. The functional heterogeneity of macrophages is reflected by a great phenotypical diversity. Depending on their tissue site and activation status a range of macrophages, from resting resident (e.g., Kupffer cell, alveolar macrophage) to fully activated inflammatory macrophages can be found [1]. To exercise their function macrophages express various receptors such as Fc-, complement-, and scavenger receptors, adhesion molecules, and receptors for soluble mediators such as cytokines, chemokines, prostaglandins, and growth factors. The expression of the different receptors varies depending on the tissue localization and activation status of the macrophage [2, 3]. Studies on the working mechanisms and the description of the intracellular signal pathway in macrophages have been hampered by the fact that often more than one of these receptors is involved in the binding of one ligand. The best-studied examples, the responses to lipopolysaccharide (LPS) [4] and macrophage colony-stimulating factor [5], reflect the enormous complexity of this system.

Recently, CD163, formerly referred to as M130 [6] or RM3/1 antigen [7], has been identified as a receptor exclusively expressed on monocytes and macrophages [8]. CD163 belongs to the group B scavenger receptor cysteine-rich (SRCR) superfamily, which also includes CD5 and CD6 primarily expressed on B and T lymphocytes, and WC1 present on $CD4^{-}8^{-}$ γδ T lymphocytes [9]. Little is known about the function of this family of receptors but a role in intercellular contacts has been suggested. Studies on CD6 indicated that the SRCR domains might be responsible for the cellular interaction by binding to its ligand CD166 on activated leukocytes [10]. CD163 is 130-kDa glycoprotein with an amino-terminal signal element, 9 SRCR-domains, one transmembrane element, and a short cytoplasmic tail. At least four splice variants have been described that differ mainly in the cytoplasmic tail [8, 11]. A recent study showed that CD163 was involved in the adhesion to endothelial cells [12].

So far little is known about the role of CD163 in the regulation of macrophage effector function. This study describes aspects of the regulation of the CD163 expression on monocyte-derived macrophages by the use of a novel monoclonal antibody against CD163. In addition, the putative role of this receptor in macrophage function, in particular production of pro-inflammatory cytokines, is investigated, as well as the pathway used by CD163 for intracellular signaling. Cross-linking of this receptor with the monoclonal antibody induced a cascade of intracellular signals that involved a tyrosine kinase-dependent calcium mobilization, inositol (1,4,5) triphosphate (IP_3) and IP_4 (IP_x) production, interleukin-6 (IL-6), and granulo-

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cyte macrophage colony-stimulating factor (GM-CSF) secretion. IL-1β production was induced as well, albeit in a different tyrosine kinase-independent fashion.

MATERIALS AND METHODS

Development of monoclonal antibody

Leukocytes were isolated from fluids that were sampled from the pleural cavity of patients with idiopathic spontaneous pneumothorax. The macrophage content varied from 60 to 90% as determined by May-Grünwald-Giemsa staining and the contaminating cells were eosinophilic and neutrophilic granulocytes. The cells were stored in liquid nitrogen until further use. A BALB/c mouse was immunized by repeated injections of 2.5×10^7 cells using an immunization protocol as described previously [13]. Spleen cells were then fused with the mouse myeloma cell line SP20 and the resulting hybridomas were grown under hypoxanthine aminopterin thymidine selection. Hybridoma supernatants were collected and screened for specific reactivity to human spleen macrophages and pleural macrophages with immunohistochemistry (see below) and subcloned by limiting dilution plating. Hybridoma supernatants or purified biotin-labeled antibody were used in all experiments. The EDHU1-Ab, one of the developed monoclonal Ab, was of the IgG1 subclass as determined by immunohistochemistry with isotype-specific conjugates.

Cell isolation

Leukocytes were isolated by loading heparinized blood that had been diluted with phosphate-buffered saline (PBS; 1:1, v/v) on lymphoprep (Nycomed, Oslo, Norway). The gradient was left on the bench for 45–60 min and the hypodense fraction containing the leukocytes was isolated. The remaining erythrocytes were removed by a hyposmolar shock in a hypotonic salt solution after which the cells were washed twice.

For the isolation of monocytes, peripheral blood mononuclear cells (PBMC) were isolated from buffy coats on a lymphoprep gradient by centrifugation (600 *g*). Lymphocytes were partly removed by rosetting to sheep red blood cells (Biotrading, Mijdrecht The Netherlands) and monocytes were further purified by adherence of the non-rosetting cells for 2 h on Petri dishes (B & D, Franklin Lakes, NY) in medium containing RPMI-1640 (GIBCO, Bio-Cult, Irvine, Scotland) supplemented with 2 mM L-glutamine (Gibco), 10% heat-inactivated fetal calf serum, 50 mM mercaptoethanol (Sigma), 50 U/mL penicillin (GIBCO), 100 μg/mL streptomycin (GIBCO), and 50 μg/mL gentamicin (GIBCO). Nonadherent cells were then removed by gentle flushing with medium and the remaining adherent cells were mainly monocytes with a purity of 80–90%. For functional studies monocytes were isolated from PBMC by counter-flow elutriation (Beckman centrifuge J2-21M, rotor JE-6B, standard chamber) and this way a purity of >95% was obtained as determined by examination of cytospin preparations after a May-Grünwald-Giemsa staining [14]. In experiments on the regulation of the CD163 expression, monocytes were cultured for various periods of time in RPMI/fetal calf serum (FCS) in the presence of 10 pM–10 μM dexamethasone (DEX) (Sigma Aldrich NV/SA, Bornem, Belgium), 100 U/mL interferon-γ (IFN-γ; Endogen, Cambridge, MA), 500 U/mL IL-4 (Genzyme, Cambridge, MA), 1000 U/mL GM-CSF (Schering-Plough, Ireland), 1 μg/mL LPS (*Escherichia coli*, serotype 026-B6, Sigma), or 5 ng/mL phorbol myristate acetate (PMA; Sigma).

Monocyte-derived dendritic cells (DC) were developed by incubating the adherent non-rosetting monocytes in medium containing 500 U/mL rIL-4 and 1000 U/mL rGM-CSF. On day 6 the cells had become nonadherent and were collected [15]. Viability was always above 95%.

Mature blood DC were isolated by the method described by Freudenthal et al. [16]. In short, lymphocytes were removed from the mononuclear fraction by red blood cell rosetting. Cells were cultured overnight on Petri dishes. The nonadherent fraction was cultured for another hour on Petri dishes to remove other adherent cells. Fc-positive monocytes were depleted by Fc-receptor panning and nonadherent cells were layered on hypertonic 12.5% metrizamide, dissolved in medium (Nycomed), and sedimented at 600 *g* for 10 min at room temperature. The interface was harvested and contained a DC-enriched fraction (30–60% purity).

Immunohistochemistry

Biopsies of normal human tissues (cerebellum, cerebrum, liver, lung, lymph node, skin, spleen, tonsils, thymus) of at least two different individuals per tissue type were snap frozen in liquid nitrogen and stored at −70°C. Most tissues were obtained after surgical procedures except brain tissue, which was collected *post-mortem* (Netherlands Brain Bank, Amsterdam, The Netherlands). Frozen sections of 8–9 μm were prepared and air dried on gelatin-coated slides. Cytospin preparations of DC were made using 2×10^4 cells per slide. Sections or cytospin preparations were fixed for 10 min in acetone and washed in PBS.

Immunoperoxidase

Preparations were incubated with the first monoclonal antibody in PBS/1% bovine serum albumin (BSA) for 45–60 min at 20°C. Subsequently, preparations were washed twice and incubated with horseradish peroxidase-conjugated rabbit anti-mouse Ig (DAKO A/S, Glostrup, Denmark) for 45–60 min. 3,3'-Diaminobenzidine (Sigma) + H_2O_2 was used as chromogen and peroxidase substrate.

Alkaline phosphatase

Sections were incubated with biotinylated EDHU1-Ab in PBS/1% BSA for 45–60 min, washed twice, and incubated with streptavidin coupled to alkaline phosphatase (Vector Laboratories, Burlingame, CA). Naphtol-AS-BI (Sigma) was used as substrate. Acid phosphatase activity in macrophages and DC was demonstrated using the method according to Burnstone [17] and naphtol-AS-BI was used as substrate. Double-staining procedures involved a sequential peroxidase and acid or alkaline phosphatase staining. All preparations were counterstained with hematoxylin immediately after immunochemistry and embedded in aquamount.

Flow cytometry

Cells were resuspended in PBS/0.1% BSA and incubated with the EDHU1-Ab or a mouse anti-human mannose receptor Ab [18] for 45 min at 4°C. The isotype control (IgG1) used in each experiment was a mouse anti-rat antibody (ED8) with no cross-reactivity to a human antigen. Cells were washed twice and incubated for 45 min with fluorescein isothiocyanate (FITC)-labeled $F(ab')_2$ fragments of goat anti-mouse antibody (DAKO) at 4°C. Cells were washed and the fluorescence was measured on a FACScan flow cytometer (Becton-Dickinson, San Jose, CA).

Electron microscopy

Monocyte-derived cells were fixed in 2% paraformaldehyde and 0.5% glutaraldehyde (Merck, Darmstadt, Germany) at room temperature. Sucrose-infused cell samples were frozen in liquid nitrogen and ultra-thin sections were prepared [19, 20]. Sections were labeled with EDHU1-Ab followed by colloidal gold particles coupled to protein A [21]. The sections were examined using a CM 100 electron microscope (Philips, Eindhoven, The Netherlands).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting

Preparation of cell lysates, the SDS-PAGE, and the Western blot were performed as described [11]. In short, the HEK cells were incubated with 1–10 mM Pefabloc (Boehringer Mannheim, Mannheim, Germany), 1 mM $CaCl_2$, 1 mM $MnCl_2$, and octylthioglucopyranoside (Sigma) at a protein-detergent ratio of 0.2 for 30 min at room temperature under gentle rotation. After centrifugation, the supernatant was collected and the protein extract was separated by preparative SDS-PAGE under nonreducing and nondenaturing conditions according to Leammli with the use of an 8% running gel [22]. For Western blotting, the gel was transferred to a Protran nitrocellulose membrane (0.45 μM, Schleicher & Schuell, Dassel, Germany) in a semi-dry blotting unit (Höfer/Pharmacia, Freiburg, Germany) applying a 0.8-mA/cm² membrane. Subsequently, proteins were detected immunochemically. Nonspecific binding was blocked by incubating the membrane with 1% skim milk powder in PBS for 1 h. After washing with PBS, the membranes were incubated for 1 h with the primary antibody (12 μg/mL) in TBS buffer (50 mM Tris-HCl, pH 7.6, 145 NaCl) containing 0.1% BSA. Subsequently, the membrane was washed in TBS containing 0.05% Tween-20 and incubated with the alkaline phosphatase-

labeled secondary goat anti-mouse IgG1 (75 ng/mL, in TBS, 1 h). After washing the membrane with TBS-Tween and HP buffer (100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 50 mM $MgCl_2$), colorimetric detection was performed by addition of 0.15 mg/mL BCIP and 0.3 mg/mL NBT in HP buffer.

EDHU1-Ab-induced intracellular signaling and cytokine production

Monocyte-derived macrophages were obtained by culturing elutriated monocytes in RPMI/FCS in 5% CO_2 at 37°C for 4–6 days in the presence of DEX (0.1 μM) and GM-CSF (1000 U/mL) in order to obtain maximal CD163 expression.

Measurement of free intracellular calcium

For the Ca^{2+} assays, macrophages were loaded with 1 μM Fura-2 acetoxymethyl ester (Molecular Probes, Eugene, OR) for 30 min, at 37°C, washed twice, and diluted to 1×10^6 cells/mL in physiological salt solution (PSS; NaCl 115 mM, KCl 5 mM, KH_2PO_4 1 mM, $MgSO_4$ 1 mM, glucose 10 mM, HEPES 25 mM, $CaCl_2$ 1.25 mM, BSA 0.1%, pH 7.4), and used immediately. Fluorescence recordings were made in a continuously stirred cuvette at 37°C in a fluorescence spectrophotometer (Shimadzu, Shimadzu-Benelux, 's-Hertogenbosch, The Netherlands). Data were recorded as the relative ratio of fluorescence emitted at 510 nm after sequential excitation at 340 and 380 nm. Each run was individually calibrated and intracellular calcium was quantified using the equation described by Grynkiewicz et al. [23] after a modification of the equation according to Scanlon et al. [24]; *N*-formyl-Met-Leu-Phe (fMLP, Sigma) was used as a positive control.

IP_x measurement

Cells were grown for an additional 24 h in Teflon wells in inositol-deficient DMEM (GIBCO) with 10% FCS, 1000 U/mL GM-CSF, 0.1 μM DEX, and supplemented with 1 μCi/mL [*myo*-^{3}H]inositol (Amersham Life Sciences, Buckinghamshire, UK) and maintained in culture in 5% CO_2 at 37°C. Cells were centrifuged, washed once, and resuspended in DMEM/FCS containing 25 mM HEPES and 10 mM LiCl (pH 7.4), and seeded into 24-well tissue culture plates (1 to 2×10^5 cells/well) that had been pre-coated overnight with purified EDHU1-Ab or the control Ab (ED8). Next, blockers and/or stimulants were added as indicated. After a 60-min incubation in 5% CO_2 at 37°C, supernatants were removed, cells were sonicated in ice-cold chloroform/methanol (1:1, v/v), and IP_x accumulation was measured using ion-exchange chromatography as described previously [25].

Cytokine production

Cells were washed twice and resuspended in medium at a concentration of 10^6 cells/mL. Cells were seeded at a concentration of 2×10^5 cells/mL into 96-well tissue culture plates that had been pre-coated with 10 μg/mL EDHU1-Ab (as indicated above) or control Ab (ED8) and cultured in triplicate. The supernatants were harvested after 24 h of culture and stored at −20°C until further use. IL-1β, IL-6, and GM-CSF concentrations were determined by ELISA (all from BioSource International Inc.). The lower detection limits of the cytokine enzyme-linked immunosorbent assays (ELISAs) ranged from 1 to 10 pg/mL.

In the above experiments several inhibitory agents of signal transduction pathways were used, namely genistein and calphostin C (Biomol, Plymouth Meeting, PA) and H7 (Calbiochem, La Jolla, CA) and they were added 5–15 min before cross-linkage of the CD163.

RESULTS

Tissue distribution of the antigen recognized by the EDHU1-Ab

The vaccination protocol with pleural macrophages resulted in a set of monoclonal antibodies including EDHU1-Ab. The expression of the antigen that is recognized by the EDHU1-Ab was studied on various tissues, on blood leukocytes and on monocyte-derived macrophages (**Table 1**).

TABLE 1. Tissue Distribution of EDHU1-Antigen (CD163)

Tissue type	EDHU1 antigen (CD163)
Blood	
Monocytes	−
Granulocytes	−
Lymphocytes	−
Erythrocytes	−
Platelets	−
Lymphoid tissue	
Lymph node	+ (medullary + perifollicular macrophages)
Spleen	+ (red pulp macrophages)
Thymus	+ (macrophages in the medulla and cortex, specially at the border between the cortex and medulla)
Tonsil	+ [perifollicular, (epidermis)]
Other	
Liver	+ (Kupffer cell)
Lung	+ (alveolar and interstitial macrophage)
Brain	+ (perivascular and meningeal macrophage)
Skin	+ (dermal macrophage)
Monocyte-derived macrophages	+
Myeloid cell lines	
Monomac 6	−
THP-1	−
U937	−
Dendritic cells	
Langerhans cell	−
Mature blood DC	few
Monocyte-derived DC	−

Data of tissue distribution of at least two different staining procedures are presented.

Tissue sections predominantly showed staining on resident tissue macrophages such as red pulp macrophages in the spleen, Kupffer cells in the liver, and interstitial macrophages and alveolar macrophages (AM) in the lung. In addition, the antigen was present on medullary and perifollicular macrophages in the lymph node, perifollicular macrophages in the tonsils, medullary and cortical macrophages in the thymus, especially those situated at the border between the medulla and the cortex, and perivascular and meningeal macrophages in the central nervous system.

Blood cells were tested by FACS analysis in order to determine the expression on freshly isolated myeloid and lymphoid cells. Neither erythrocytes nor any of the leukocytes showed a fluorescence signal above that of the isotype control, indicating that the cells did not express any antigen or expressed it at a very low level. To ascertain this finding, expression was determined on fresh leukocytes from 15 healthy volunteers and FACS analysis confirmed the absence of the antigen on monocytes, lymphocytes, and granulocytes. The EDHU1 antigen was also not detected on the tested myeloid cell lines (U937, THP-1, Monomac-6).

DC in the T and B cell areas in the studied lymphoid organs, Langerhans cells in the skin, and monocyte-derived DC [15] did not show any expression. Furthermore, mature blood DC isolated by the method described by Freudenthal [16] were mostly negative, although a minor subpopulation (5–10%) showed a weak expression.

Immunoblotting of CD163-transfected HEK cells

In general, the tissue distribution was reminiscent of the expression of CD163 [7, 8]. Therefore, transfected HEK cells stably expressing a cDNA encoding CD163 were tested to determine whether EDHU1-Ab recognized CD163 [11]. Indeed, immunoblotting of lysates of the transfected HEK cells revealed that both the EDHU1-Ab and the RM3/1-Ab recognized a 130- to 140-kDa protein, i.e. CD163 (**Fig. 1**, lanes A), whereas they did not recognize a protein in the non-transfected HEK cell lysates (lanes B).

Expression regulation of the antigen recognized by the EDHU1-Ab

The immunohistochemical analysis demonstrated that the EDHU1 antigen expression is mainly restricted to mature macrophages. To investigate the regulation of expression, monocytes were cultured *in vitro* in the presence of several factors with known effects on monocyte differentiation. The regulation of the expression was compared to that of the mannose receptor.

A marked induction was seen when DEX was added to the monocyte cultures, whereas expression could not be induced by several cytokines (**Fig. 2A**). IFN-γ and IL-4 had little or no effect on the expression. Prostaglandin D_2, E_2, and J_2 also did not show any effects (data not shown). In contrast, the macrophage-activating agents LPS and PMA had a modest stimulatory effect. The antigen was expressed at a low level after 2 or more days of culture in medium alone (Fig. 2B). The regulation of expression was different from that of another glucocorticoid-inducible antigen, the mannose receptor, which was clearly up-regulated by DEX, GM-CSF, IL-4, or the combination of IL-4 and GM-CSF.

Next, time- and dose-dependency of the DEX-induced EDHU1 antigen expression were studied. Although monocytes were EDHU1-negative at day 0 of culture, the presence of DEX induced a high expression that reached its maximum at day 6 (Fig. 2B). The effect was dose-dependent and DEX enhanced the expression at a concentration between 10 μM and 1 nM (Fig. 2C). Optimal expression was found at the concentration of 0.1–1 μM. A brief incubation of monocytes for 2 h followed by washing did not influence the expression (data not shown) and indicated that the presence of glucocorticoids for a longer period was essential. The DEX-induced expression was inhibited in a dose-dependent fashion by the addition of the glucocorticoid receptor antagonist RU486 (Fig. 2D).

Because IFN-γ and IL-4 were observed to inhibit the spontaneous expression of the EDHU1 antigen in some experiments, it was speculated that these cytokines also were able to inhibit the DEX-induced expression. Unexpectedly, the combination of DEX with LPS, PMA, IL-4, or GM-CSF had a synergistic effect on the expression (Fig. 2E), whereas IFN-γ or the combination of IL-4 and GM-CSF did not clearly affect the DEX-induced expression. Again, the mannose receptor showed different patterns of expression regulation.

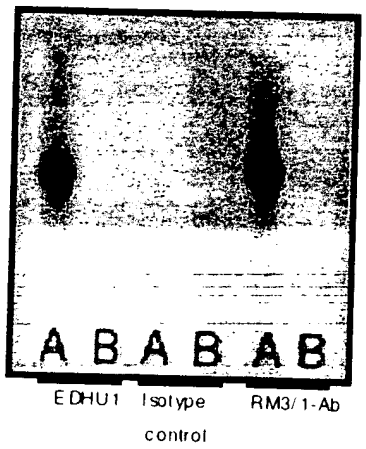

Fig. 1. The EDHU1-Ab recognizes human CD163 Western blot of lysates from stably transfected CD163 (A) and non-transfected (B) HEK cell lines immunostained with EDHU1-Ab, isotype-Ab, and RM3/1-Ab.

Subcellular CD163 distribution on AM

To assess the ultrastructural distribution of the CD163, AM were studied by immuno-electron microscopy. AM, isolated from a bronchoalveolar lavage, are large cells of 20–25 μm in diameter that show membrane ruffling and contain many large phagolysosomes (see **Fig. 3**). The CD163 was mainly detected at the plasma membrane and to a lesser extent in small vesicles in the cytoplasm compartment, whereas phagolysosomes did not show any staining. Monocyte-derived DC analyzed in parallel did not show any CD163 expression.

Cross-linking of CD163 with the EDHU1-Ab induces intracellular signaling and cytokine production

To study the potential relevance of CD163 in signaling and activation, the molecule was cross-linked at the cell surface of monocyte-derived macrophage with the EDHU1-Ab. The appropriate macrophages were generated by culturing elutriated monocytes for 4–6 days in the presence of DEX and GM-CSF to induce an optimal antigen expression.

Cross-linking of the antigen on the tissue culture plates with the EDHU1-Ab resulted in the production and secretion of IL-1β, IL-6, and GM-CSF (**Table 2**). The isotype control antibody did not induce any cytokine production. The IL-6 production was higher than that induced by the otherwise potent stimulus IFN-γ/LPS. GM-CSF and IL-1β were not detected in the culture supernatants of macrophages after the latter stimulus. In contrast, the $CD163^-$ monocyte-derived DC generated from cells from the same donor did not produce any cytokine in the presence of the EDHU1-Ab, although this cell type was able to produce large amounts of IL-6 and IL-1β after stimulation with LPS and IFN-γ (data not shown). Data from the literature suggest the involvement of calcium in the cytokine production by macrophage cell lines [26] and we analyzed CD163-triggered Ca^{2+} mobilization. $CD163^+$ macrophages were loaded with the Ca^{2+} indicator Fura-2 and the EDHU1-Ab was added to the cell suspension to induce cross-linking, after which the calcium mobilization was detected by fluorospectrophotometry. A transient signal was detected at 40–60 s after the addition of the Ab (**Fig. 4A**). In addition, the induced

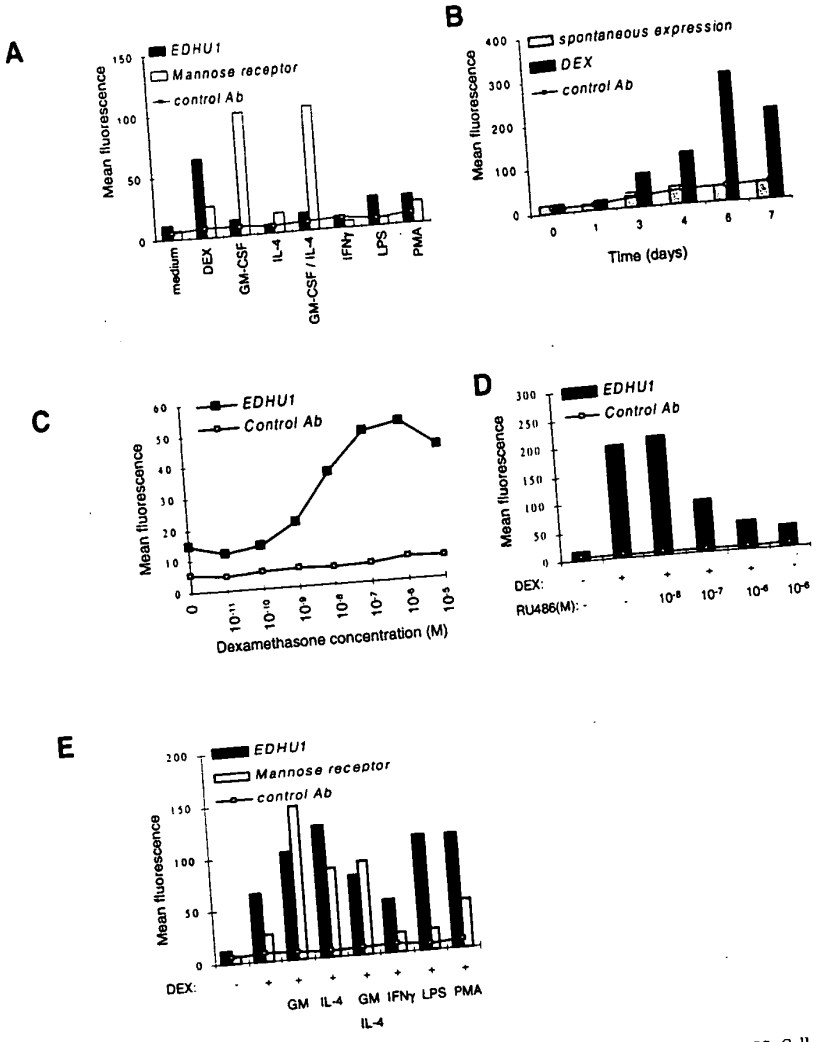

Fig. 2. Expression regulation of EDHU1 antigen. Adherent monocytes were cultured in medium containing FCS. Cells were harvested 0–7 days after the start of culture and EDHU1 antigen and mannose receptor (MR) expression was determined by flow cytometry. (A) The regulation of expression was studied by adding the following cytokines and activating agents to the monocyte cultures (2 days): DEX, GM-CSF, IL-4, the combination of GM-CSF and IL-4, IFN-γ, LPS, or PMA. (B) Time dependency of the antigen expression was studied by culturing monocytes for 0–7 days in the presence or absence of 0.1 μM DEX. (C) Dose dependency was studied by culturing monocytes for 2 days in the presence of graded doses of DEX. (D) The direct influence of glucocorticoids on the expression was studied by incubating monocytes for 2 days with DEX and the glucocorticoid receptor antagonist RU486. (E) Monocytes were cultured for 2 days with a combination of DEX and the different agents to assess the effect of co-incubation. Representative experiments of three are given.

mobilization was fully inhibited by pre-incubating the cells with the protein tyrosine kinase (PTK) inhibitor genistein (see inset). The control antibody did not have any influence on the intracellular calcium levels. The calcium mobilization was different from that generated by fMLP, which induced an immediate transient signal that already could be detected within seconds. Genistein did not affect the fMLP-induced calcium mobilization.

The slow sustained calcium release suggested the involvement of IP_3 and IP_4 (together referred as IP_x) and therefore the effect of cross-linking of CD163 on the IP_x production was studied. Incubation of $CD163^+$ macrophages for 1 h on EDHU1-Ab-coated plates resulted in IP_x accumulation (Fig. 4B) that was dependent on the concentration of coated Ab. Neither the control Ab nor LPS had any effect on the IP_x production. Again, genistein was able to block the signal in a concentration-dependent fashion.

Because cross-linking clearly induced cytokine production and a PTK-dependent signal into the cell, it was postulated that these two aspects were related. Indeed, genistein inhibited the production of IL-6 and GM-CSF ($P < 0.001$) yet the production of IL-1β could not be blocked (Fig. 4C). This indicated that the cells were viable and that other PTK-independent regulatory pathways might become activated as well. The cyclic nucleotide-dependent protein kinase inhibitor H7 and the more specific protein kinase C inhibitor calphostin C did not affect the IL-6 and GM-CSF production but first decreased the IL-1β production by 50% ($P < 0.05$).

Fig. 3. Distribution of CD163 on the alveolar macrophage. The cellular distribution of the antigen recognized by the EDHU1-Ab (CD163) bound to colloidal gold particles was studied by transmission electron microscopy (original magnification ×27,000). The inset shows a typical alveolar macrophage (original magnification ×1200).

DISCUSSION

In this study a novel monoclonal antibody (EDHU1) against CD163 was described. This SRCR-superfamily receptor is selectively expressed on a subset of resident macrophages. Activation of CD163 on the cell surface, by cross-linking with EDHU1-Ab, induced a cascade of intracellular signals involving PTK-dependent calcium mobilization, IP_x production, IL-6, and GM-CSF secretion. IL-1β production was also induced but in a different PTK-independent fashion.

The monoclonal antibody described in this study, EDHU1-Ab, recognized an antigen exclusively expressed by a specific subset of macrophages and in addition the antigen was up-regulated by glucocorticoid. The data suggested that the EDHU1-Ab recognized CD163 and indeed this was confirmed by immunoblotting of CD163-transfected HEK cells. However, some minor discrepancies were observed with data from the literature. First, in some studies monocytes were found to show a low expression of CD163 [7, 27], whereas in the present study no expression of CD163 was observed. Second, phorbol esters inhibited the CD163 expression [8, 11], whereas according to our data they slightly induced, and in combination with glucocorticoids, even markedly induced CD163 expression. At present these disagreements are not fully understood, but a likely explanation might be the differences in the cell isolation procedures.

TABLE 2. The Cytokine Production After EDHU1 Antigen Cross-linking

	IL-1β	IL-6	GM-CSF
Control Ab	<5	<20	<40
EDHU1-Ab	642 ± 63	638 ± 88	725 ± 106
LPS + IFN-γ	<5	205 ± 7	<40

The cytokine concentration (pg/mL), produced by monocyte-derived macrophages 2×10^5 cells/mL (in triplicate), was measured 24 h after cross-linking of the antigen on the tissue culture plate with the EDHU1-Ab or after stimulation with LPS and IFN-γ. ED8 was used as an isotype control antibody. A representative experiment out of three is given.

Despite the large amount of data on the effects of glucocorticoids on the immune response, little is known about its influence on the macrophage. Available data mainly emphasize its suppressive effects on cytokine production and surface receptor expression [28]. However, some studies have shown effects on macrophages that might lead to the increase of certain macrophage functions by inducing receptor expression such as is the case for the mannose receptor [29] and sialoadhesin [30]. In this respect, it is interesting to note that the present data and previous studies [8] show that expression of the CD163 on monocyte-derived macrophage is also under the control of glucocorticoids. Another interesting feature was the observed synergetic effect of glucocorticoids in combination with a panel of cytokines and stimulators. The glucocorticoid-induced expression of CD163 was enhanced when combined with cytokines or stimulating agents such as LPS and PMA, whereas these factors alone were poor inducers or had a modest inhibitory effect (e.g., the cocktail of IL-4 and GM-CSF). Similar synergistic mechanisms were also observed regarding expression of the mannose receptor [31] and for sialoadhesin [30].

Glucocorticoid-incubated macrophages only secreted relatively low amounts of IL-6 when a strong stimulus LPS/IFN-γ was given, whereas IL-1β and GM-CSF could not be detected at all. These data are in concordance with the suppressive effects of glucocorticoids on cytokine production [32]. The presented data, however, show that these macrophages fully retain their potency to produce cytokines. Whenever a ligand for CD163 is present *in vivo*, the glucocorticoid-incubated macrophages might even become significant cytokine producers as was suggested by the EDHU1-Ab cross-linking experiments.

CD163 is a member of the rapidly expanding group B SRCR superfamily [9, 10]. The function of this evolutionary highly conserved group of receptors [33, 34] is poorly understood but a role in the intercellular contact has been suggested [10, 35, 36]. The tissue distribution of CD163 on resident tissue macrophages nonetheless suggests a role for CD163 in either phagocytosis or local immune regulation, whereas a role in, for instance, antigen presentation, which mainly takes place in T and B cell areas in the lymphoid organs, was highly improbable. To acquire more information on its role in phagocytosis and adherence, blocking experiments were performed in which macrophages were pre-incubated with the EDHU1-Ab. However, no effects were shown on either the phagocytosis of different bacteria (*S. aureus*, *E. coli*) and particles (zymosan, myelin, latex, Ficoll) or the adherence to coated tissue culture plates (poly-L lysine, gelatin, serum, collagen; data not shown) giving us no indication for the involvement of CD163 in these functions. Yet, data from the literature showed a role for CD163 in adhesion of macrophages to human umbilical vein endothelial cells, suggesting a function in cell-cell interaction [12]. After ligation, the macrophages produced pro-inflammatory cytokines IL-1β and IL-6 as well as GM-CSF. CD163 can therefore have a function in the intercellular communication.

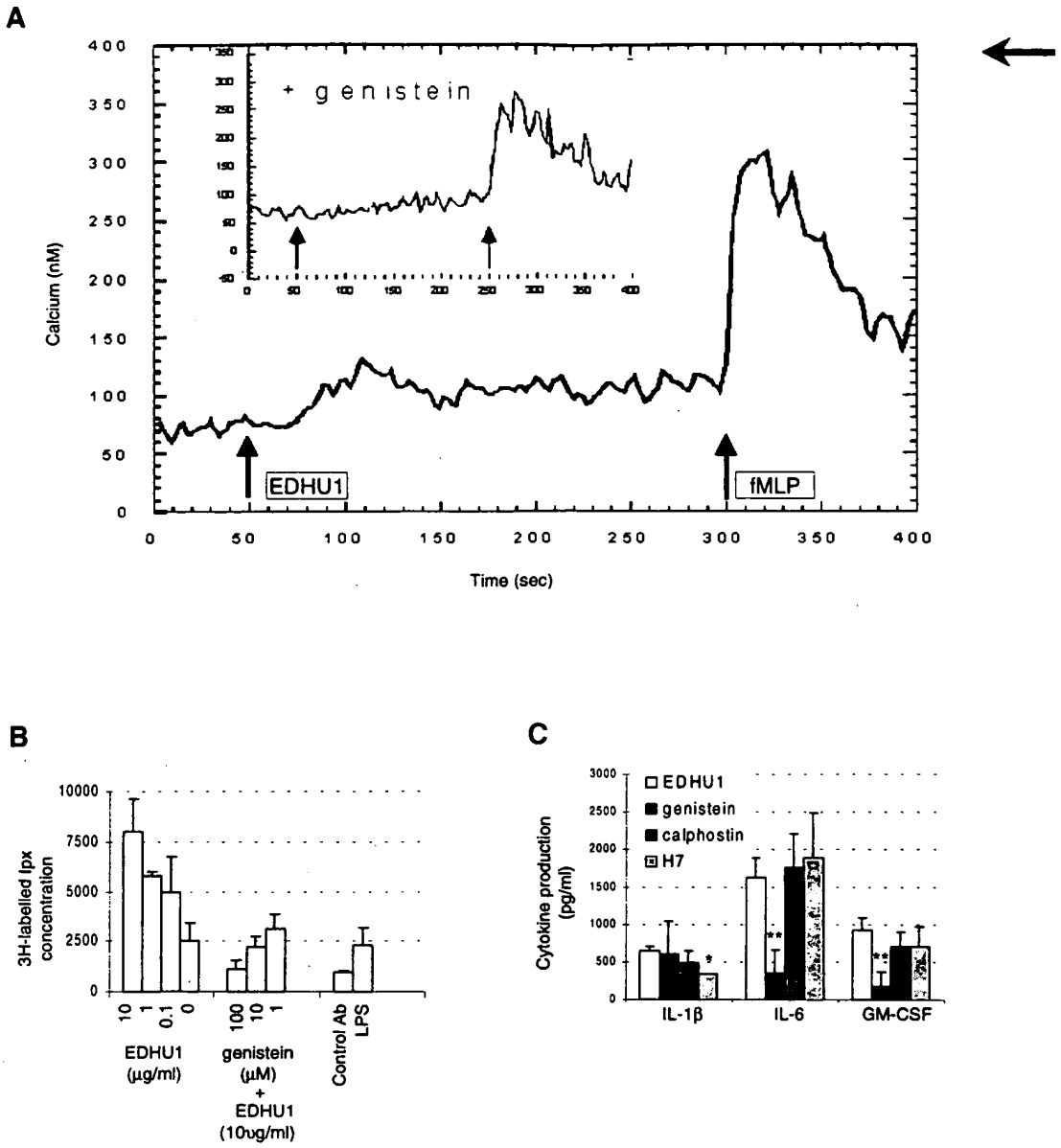

Fig. 4. Signal transduction and activation by cross-linking of CD163 with EDHU1. (A) Calcium mobilization in CD163-positive macrophages after the addition of EDHU1-Ab to the cell suspension, and the influence of genistein (100 µM; see inset). (B) IP_x production after cross-linking of the CD163 on the tissue culture plate and the influence of genistein (100 µM). (C) Cytokine production in 24 h by 2×10^5 cells/mL (pg/mL) and the influence of genistein (100 µM), calphostin C (0.5 µM), or H7 (20 µM).

The present data are the first that describe a signaling pathway via CD163. Cross-linking induced a PTK-dependent signal, which finally resulted in calcium mobilization and the induction of IP_x and cytokine production. The calcium mobilization was of a slow type, only occurring about 1 min after receptor ligation and was very different from the immediate transient response as induced by fMLP or by activation of the LPS receptor CD14 with specific Ab [26]. The PTK-dependent IP_x production indicated the involvement of phospholipase Cγ (PLCγ) [37]. On phosphorylation by an unidentified PTK, PLCγ hydrolyzes phosphatidylinositol 4,5 biphosphate, which results in the production of diacylglycerol and IP_3. Once diacylglycerol and IP_3 are formed, other inositol phosphates are generated that can induce the mobilization of calcium as well, yet IP_3 is the dominant second messenger for intracellular calcium release [38]. The PTK-dependent signal finally led to the production of IL-6 and GM-CSF. A similar PTK dependency has been shown for the LPS-induced IL-6 production by monocytes [39]. In addition, CD163 ligation also induced a PTK-independent signal, as can be concluded by the lack of inhibition of IL-1β production by genistein. A cyclic nucleotide-dependent protein kinase might be involved because H7 could partly block the IL-1β production and this is also in concordance with LPS data from the literature [40]. At the same time the lack of response after calphostin C incubation suggests that protein kinase C is not involved.

It is currently unknown how CD163 ligation can transduce the signal into the cell. The cytoplasmic domain CD163

consists of 49 amino acids, although some splice variants show longer domains [6, 8]. It contains one to three tyrosine residues depending on the splice variants, and possesses no known PTK motifs. Therefore the exact mode by which CD163 transduces its signal into the cell and induces PTK-sensitive and -insensitive pathways that lead to the production of the different cytokines will have to be determined.

In summary, the SRCR-superfamily receptor CD163 was identified as a receptor that might play a role in immunoregulatory aspects of the resident macrophage. The protein is mainly expressed on mature resident macrophages and not on monocytes. Activation of this protein by a specific monoclonal antibody results in signaling and ultimately in the secretion of the cytokines IL-1β, GM-CSF, and IL-6. These observations therefore not only suggest a novel role for SRCR superfamily receptors in cell signaling but also identify mature macrophages as a potential source of pro-inflammatory cytokines during an immune response, if activated by an endogenous natural CD163 ligand.

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Topoll et al. J. Peridontal Res. 24: 106-112 (1989).

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Phenotypic dynamics of macrophage subpopulations during human experimental gingivitis

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Topoll HH, Zwadlo G, Lange DE, Sorg G: Phenotypic dynamics of macrophage subpopulations during human experimental gingivitis. J Periodont Res 1989; 24: 106–112.

The purpose of the present study was to investigate whether functionally different macrophages are present in clinically healthy gingiva and during human experimental gingivitis. Eight male probands were introduced to an oral hygiene program until all reached mean Plaque and Gingival Index scores approaching zero. During the following 19 days all oral hygiene was abandoned. At d −14, 0, 2, 4, 7, 11 and 19 clinical indices and gingival biopsies were taken. Cryostat sections were incubated with monoclonal antibodies against mature macrophages (25F9), inflammatory macrophages (27E10) and anti-inflammatory macrophages (RM 3/1). Positive cells were counted in the inflammatory infiltrate (IF) and the connective tissue (CT). At d −14 elevated numbers of 27E10-positive cells were observed which decreased significantly at d 0 ($p < 0.018$) and increased again at d 19 ($p < 0.026$). Significant differences in the number of RM 3/1-positive cells were found between d 0 and d −14, 2, 4 and 7 ($p < 0.05$) while no differences in the number of 25F9-positive cells were observed throughout this study. It was concluded that experimental gingival inflammation is characterized by the appearance and disappearance of functionally different macrophage subpopulations.

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Introduction

The inflammatory infiltrate of gingivitis and marginal periodontitis lesions has been investigated by qualitative and morphometric point-counting procedures (1, 2, 3). Healthy gingiva and inflamed gingiva of gingivitis and periodontitis lesions were classified into 5 different stages based on the distribution and appearance of polymorphonuclear leukocytes, lymphocytes, plasma cells, marcrophages and fibroblasts (4). In recent years it has been shown that macrophages and lymphocytes are not a homologous cell population but can be differentiated into biologically active subpopulations. Therefore, using monoclonal antibodies, different subsets of T cells could be identified in the inflammatory infiltrate of gingivitis (5, 6) and periodontitis lesions (7, 8, 9, 10, 11).

Numerous studies point out the importance of macrophages in inflammatory reactions (12, 13). Macrophages can act as stimulators of the T-cell response (14, 15); they are capable of secreting immunoregulatory proteins (16, 17) and can react to hormones and lymphokines (18). As effector cells they exert phagocytic and cytotoxic activity on bacteria and tumor cells (19, 20). Different macrophage subsets might therefore be involved in the inflammatory reaction during acute or chronic gingivitis and various forms of marginal periodontitis. The aim of the present study was to investigate whether functionally different macrophage subsets are present in clinically healthy gingiva and during experimental gingivitis.

Material and Methods

Clinical procedures

The experimental gingivitis model according to Löe *et al.* (21) was used. The study was approved by the Human Subject Research Committee of the University of Münster. Eight healthy 25 – to 31-year-old (mean age 27.6) male probands participated in the study. All subjects presented no radiologic evidence of alveolar bone loss. Probing depths and recession did not exceed 3 mm.

For a period of 2 wk (d −14) before the onset

of experimental gingivitis, all probands were introduced to a prophylaxis program consisting of repeated oral hygiene instructions and removal of supra- and subgingival deposits until every proband reached mean Plaque and Gingival Index scores approaching zero. During the following 19 d all oral hygiene was abandoned in order to allow plaque formation on all teeth. No restrictions concerning diet were imposed. After d 19 all teeth were professionally cleaned and the probands started their daily oral hygiene again. The oral cleanliness and the gingival conditions were evaluated 2 wk after the reinstitution of oral hygiene.

Before the hygiene phase (d −14) and at d 0, 2, 4, 7, 11 and 19 the following clinical parameters were assessed. Plaque accumulation was scored according to the criteria of the Plaque Index (PI) (22). The gingival status was determined by a modified Gingival Index (23) without probing (grade 0 = healthy gingiva, grade 1 = redness and initial swelling of the gingiva, grade 2 = moderate inflammation, redness and swelling, grade 3 = severe inflammation, severe redness and swelling).

Bleeding within 30 s after probing with a standardized force of 0.25 Newton (probe tip diameter 0.49mm)* was evaluated according to the following index scores 0 = no bleeding, 1 = minimal bleeding, 2 = moderate bleeding, 3 = severe bleeding.

All measurements were assessed at 6 different locations for every tooth present (mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual) and were carried out by one investigator (H. T.).

Immunohistology

On each experimental day biopsies of the gingival margins were taken from each proband (a total of 56 biopsies). Local anesthesia was obtained by infiltrating the vestibular fold apical to the predetermined biopsy sites with approximately 0.3 cc Xylocain containing 0.0002% Epinephrine. The biopsies were excised using two vertical, parallel incisions approximately 3 mm apart and a horizontal incision approximately 1 mm apical to the level of probe penetration. The specimens were removed with minimal trauma, oriented with tissue Tec on a metal container perpendicular to the long axis of the tooth, then immediately snap-frozen in liquid nitrogen and stored at −80°C. Approximately 4–5 μm-thick sections were cut with a cryostat in a buccolingual plane (SLEE Mainz, FRG), airdried, fixed for 10 min in acetone and processed for the indirect immunoperoxidase technique (24). Before incubation of the sections with 50% normal goat serum to inhibit nonspecific binding, endogenous peroxidase was inactivated by incubating the sections for 10 min in 0.4% sodium azide with 0.1% H_2O_2 in PBS. Sections were incubated with the monoclonal antibodies (see below) for 30 min using nonspecific mouse IgG (Dianova, Hamburg, FRG) as negative control. After washing in PBS, sections were exposed to peroxidase-conjugated goat F(ab') anti-mouse IgG for 30 min. Bound peroxidase was developed in the dark with 3-aminoethyl-4 carbazole (Sigma, Deisenhofen, FRG) for 10 min, followed by counter-staining with Mayer's hemalaun and embedding in Aquamount mountant (BDH Chemicals, Poole, U. K.). For each antibody, three sections at least 30 μm apart were evaluated.

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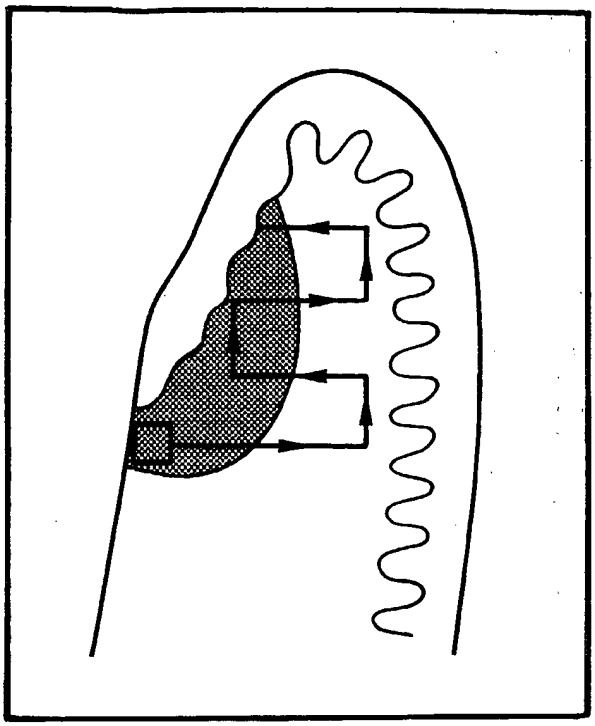

Fig. 1. Sixteen randomly selected fields were counted moving the Weibel multipurpose test system from the apical extent of the inflammatory infiltrate coronally.

Counting the different macrophage subpopulations was accomplished using a Weibel multipurpose test system at a magnification of ×400. Sixteen randomly selected fields were evaluated while moving the grid from the apical extension of the inflammatory infiltrate coronally (Fig. 1). All cells present, as well as the cells showing positive staining to each antibody in contact with the 21 grid lines, were counted (Fig. 2). The proportions of the different macrophage subpopulations were evaluated as a percentage of the total cell population counted (25, 26). Statistical analysis included an analysis of variants and a t-test for paired data.

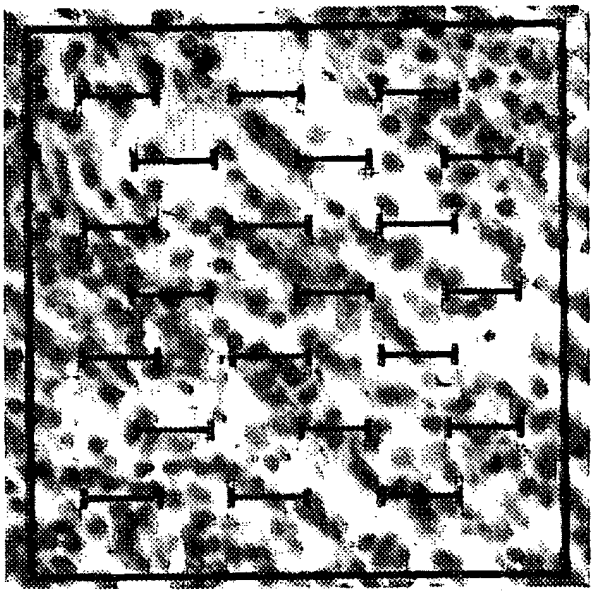

Fig. 2. The Weibel multipurpose test system superimposed on a histologic section (400×). All cells present and the positive staining cells to each monoclonal antibody in contact with the 21 grid lines were counted.

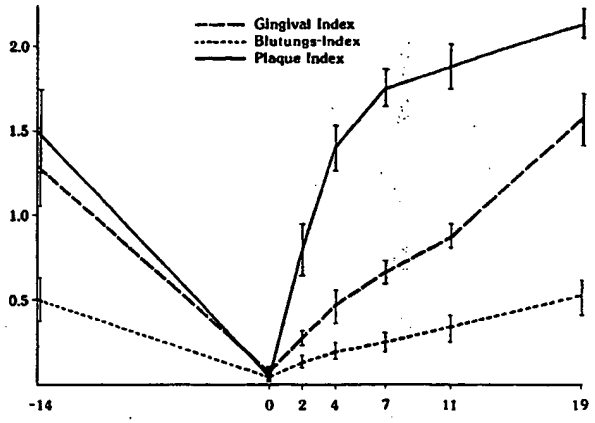

Fig. 3. Means and standard deviations of the Plaque Index scores (PII), the Gingival Index scores (GI) and the Bleeding Index scores (BI) for all probands during the experiment.

Monoclonal antibodies

In order to determine the different macrophage subpopulations the monoclonal antibodies 25F9 (24), 27E10 (28) and RM 3/1 (29) were used. The specificity of these antibodies is summarized in Table 1. In uninflamed tissue, 25F9 and RM 3/1 can be detected on resident macrophages whereas 27E10 is absent. 27E10-positive macrophages are found in the inflammatory tissue of acute urticaria, contact eczemas and erythrodermia (28). 25F9 is found on macrophages in chronic inflammatory tissues of rheumatoid arthritis, sarcoidosis and tumors (24, 25, 26). RM 3/1-positive subpopulations can be found in chronically and acutely inflamed tissues (29) (Table 1).

Additionally, these macrophage subpopulations can be differentiated by their reactions to certain biologically active substances. While 27E10 is inducible by macrophage-activating factors like interferon-8, TPA and bacterial lipopolysaccharide (LPS), the RM 3/1 antigen is downmodulated by these agents but is strongly induced by glucocorticoids. Therefore, 27E10-positive macrophages represent an inflammatory type, RM 3/1 an anti-inflammatory type, and 25F9 a resident type.

Results

At the start of the experiment, before the hygiene phase, a mean Plaque Index score of 1.5 (SD = 0.53) a mean Gingival Index of 1.2 (SD = 0.46), and a mean Bleeding Index of 0.5 (SD = 0.24) were observed (Fig. 3). At baseline (d 0) all clinical indices approached zero, PI I = 0.1 (SD = 0.05), GI = 0.1 (SD = 0.05) and BI = 0.1 (SD = 0.07) (Fig. 3). During the period of abandoned oral hygiene the mean Plaque Index score increased to 2.1 (SD = 0.19) with no statistically significant differences between any of the probands. At d 19 all individuals showed clinical signs of inflammation, although there were variations in the Gingival and Bleeding Index scores for different subjects during the period of no oral hygiene. After 2 wk of reinstituted oral hygiene and repeated oral prophylaxis all clinical indices again approached zero.

In Table 2 the Plaque Indices, Gingival Indices and Bleeding Indices for the biopsy sites of each

Table 1. Characterization on the different monoclonal antibodies 27E10 (inflammatory macrophages). 25F9 (Mature macrophages) RM 3/1 anti-inflammatory macrophages

Antibody	source		antigen	specificity
27E10	mouse	IgG 1	17 kd	10–20% of peripheral blood monocytes, macrophages only in acute inflammations, inducible by macrophage activating factors
RM 3/1	mouse	IgG 1		10–20% of blood monocytes weakly, a subpopulation of resident macrophages (eg Kupffer cells, red pulp macrophages) and macrophages in inflammatory reactions downmodulated by macrophage activating factors, inducible by glucocorticoids.
25F9	mouse	IgG 1	86 kd	no blood monocytes, a subpopulation of resident macrophages (e.g. alveolarmacrophages, white pulp macrophages), and macrophages in inflammations

Table. 2. Plaque (PII), Gingival (GI) and Bleeding (BI) Index scores during the study at the biopsy sites

Proband		exp. day						
		−14	0	2	4	7	11	19
A	PII	2	0	1	1	2	2	2
	GI	2	0	0	1	1	2	2
	BI	1	0	0	1	1	1	1
B	PII	2	0	1	1	2	2	2
	GI	2	0	0	1	1	1	2
	BI	1	0	0	1	1	2	2
C	PII	2	0	1	1	2	2	2
	GI	2	0	1	1	1	1	2
	BI	2	1	1	1	2	1	2
D	PII	2	0	0	2	2	3	3
	GI	2	0	0	1	1	1	2
	BI	3	0	0	1	1	2	2
E	PII	1	0	1	1	1	2	2
	GI	1	0	1	1	1	1	2
	BI	0	0	1	2	2	1	2
F	PII	1	0	1	1	2	2	3
	GI	2	0	0	1	1	1	2
	BI	1	1	0	1	1	1	2
G	PII	2	0	1	1	2	3	2
	GI	2	0	0	1	1	2	1
	BI	1	0	0	1	1	2	1
H	PII	2	0	1	1	1	1	2
	GI	2	0	0	1	1	1	2
	BI	1	0	0	0	1	2	2

proband and each experimental day are summarized. An increase in all three clinical parameters is found during the period of abandoned oral hygiene.

Immunohistology

The various specimens showed a range in the total number of cells counted (Table 3). Since only cells staining positive to one monoclonal antibody were counted in one specimen, the total number of cells might have influenced the absolute number of positively staining cells. Therefore, the percentage of the three macrophage subpopulations in relation to the total counted number of cells is also presented (Fig. 4, Table 3). There were statistically significant differences in the number of these subpopulations in the inflammatory infiltrate and in the connective tissue (Table 3). At d −14 all subjects showed an elevated proportion of 27E10-positive cells in the infiltrate, which decreased significantly during the phase of professional tooth cleaning ($p<0.018$) (Fig. 4). During the period of no oral hygiene the number increased with the maximal expression at d 19, although this increase was not linear. A statistically significant difference in the number of 27E10-positive macrophages was observed between d 0 and 7 ($p=0.015$) and d 0 and 19 ($p<0.026$). No differences were found in the number of these cells in the connective tissue (Table 3).

Macrophages characterized by the antigen RM 3/1 were more often detected in the inflammatory infiltrate than in the connective tissue (Fig. 4). After the oral hygiene period at d 0 an increase in the number of these cells was observed, which decreased significantly during the period of abandoned oral hygiene (Fig. 4). Statistically significant

Table. 3. Means and standard deviations of the total number of cells counted, the total number of 27E10-, RM 3/1- and 25F9-positive cells and the percentage of these subpopulations (of the total number of cells counted) in the inflammatory infiltrate (IF) and the connective tissue (CT)

exp. day	−14	0	2	4	7	11	19
27E10							
IF total cell count	195,2±25,3	151,3±31,2	173,0±23,4	207,2±37,8	220,9±34,5	191,3±27,3	240,7±34,2
IF 27 Elo pos. cells	12,3±4,6	2,4±0.7	4,6±1,7	9,9±1,9	10,7±2,7	9,9±3,2	17,7±3,2
% of total cell count	6,3±1,6	1,6±0,4	2,7±0,8	4,8±0,8	4,9±0,9	5,2±1,7	7,4±2,4
CT total cell count	60,1±9,4	56,0±16,3	62,7±14,8	67,5±12,4	57,3±13,0	66,3±19,2	73,7±13,4
CT 27 Elo pos. cells	1,0±0,3	0,4±0,2	1,1±0,3	1,6±0,2	1,5±0,5	2,2±0,4	2,3±0,7
% of total cell count	1,5±0,4	0,7±0,3	1,8±0,7	2,4±0,3	2,6±0,9	3,4±1,0	3,2±1,2
RM 31							
IF total cell count	174,3±31,0	144,1±38,0	159,4±31,2	190,8±29,4	225,0±28,7	210,3±31,3	230,4±31,5
IF RM 3/1 pos. cells	4,5±1,4	9,7±3,2	6,9±2,0	5,5±0,3	5,1±0,9	3,3±0,7	3,2±1,2
% of total cell count	2,6±0,7	6,8±1,4	4,4±0,9	2,9±0,8	2,3±0,8	1,6±0,3	1,4±0,4
CT total cell count	73,2±16,4	59,8±12,4	58,0±12,3	71,2±13,4	68,9±11,7	73,7±24,3	75,3±24,2
CT RM 3/1 pos. cells	0,5±0,4	1,1±0,3	0,6±0,1	0,8±0,1	0,5±0,1	0,6±0,1	0,5±0,2
% of total cell count	0,8±0,3	1,9±0,4	1,1±0,3	1,1±0,3	0,8±0,2	0,9±0,3	0,6±0,1
25 F9							
IF total cell count	207,4±34,7	164,2±37,1	156,3±31,2	179,8±25,4	209,8±33,6	216,0±27,9	224,3±30,4
IF 25 F9 pos. cells	3,7±0,8	1,4±0,4	1,4±0,3	2,1±0,8	3,1±0,7	5,0±0,9	5,1±1,7
% of total cell count	1,8±0,3	0,9±0,3	0,9±0,2	1,2±0,3	1,9±0,2	2,3±0,4	2,3±1,2
CT total cell count	56,3±13,7	62,4±20,7	65,8±13,4	69,0±15,6	78,3±16,7	65,4±19,2	59,3±20,4
CT 25 9 pos. cells	1,4±0,4	0,8±0,3	1,0±0,4	1,2±0,3	1,5±0,2	1,5±0,7	1,1±0,4
% of total cell count	2,5±0,8	0,1±0,0	0,2±0,0	1,8±0,7	1,9±0,3	2,3±0,8	1,9±0,6

differences in the inflammatory infiltrate were found between d – 14 and d 0, as well as between d 0 and d 2, 4 and 7 ($p < 0.05$).

The macrophage subset defined by the antigen 25F9 was more often detected in the connective tissue than in the inflammatory infiltrate (Table 3). However, no statistically significant differences in the number of these cells were noted throughout the study, either in the connective tissue or in the inflammatory infiltrate (Fig. 4).

Out of the 56 biopsies, 15 yielded a Gingival Index score of 0.25 a GI of 1 and 16 a GI of 2.

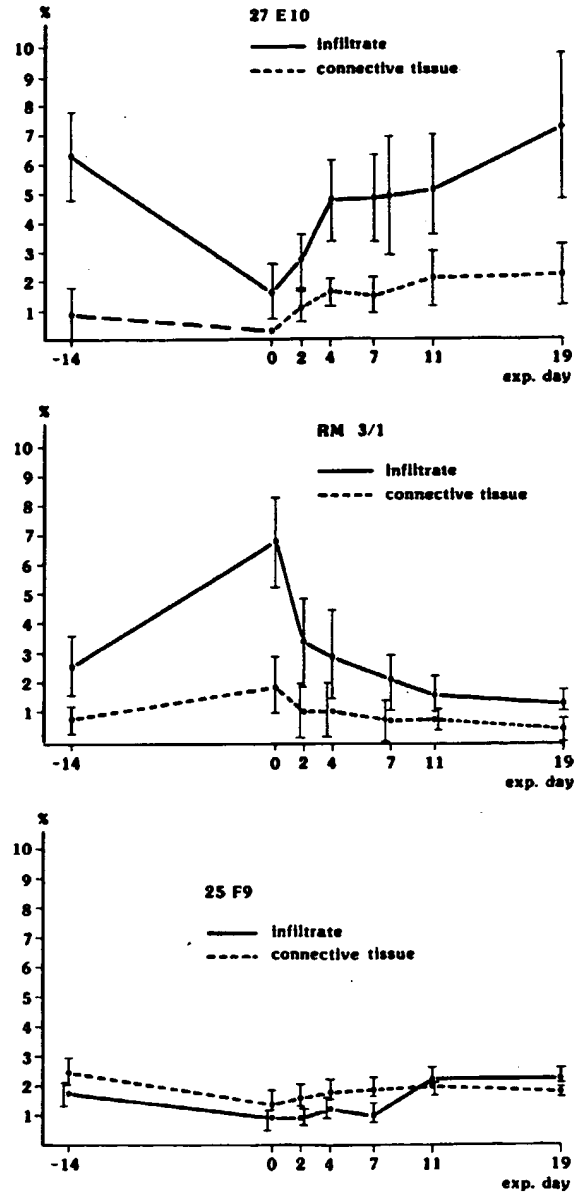

Fig. 4. Means and standard deviations of the percentage (of the total number of cells counted) of 27E10-, RM 3/1- and 25F9-positive cells in the inflammatory infiltrate (IF) and the connective tissue (CT) during the experiment.

The number of all cells in the infiltrate for the different Gingival Index scores is listed in Table 4. Clinically healthy gingiva (GI = 0) is characterized by high numbers of 25F9- and RM 3/1-positive cells and low numbers of 27E10-positive cells. With increasing GI scores a significant increase in the number of 27E10-positive cells in the infiltrate and the connective tissue was noted ($p < 0.05$). There was also a tendency toward decreased numbers of 25F9- and RM 3/1-positive macrophages in the inflammatory infiltrate and the connective tissue during developing gingivitis.

Discussion

The experimental gingivitis model (21) has been used to investigate pathologic changes in gingivitis lesions (30, 31). From these and other studies (32, 33, 34, 35, 36) different pathologic stages (healthy, initial, early and established) were defined, based mainly on the appearance and distribution of certain cell types. Since that time, new techniques have been developed to distinguish between biologically and functionally different cell subpopulations. Characterizing cells by enzyme and surface antigens, Seymour *et al.* (5, 6) observed a basically T cell-dominant inflammatory infiltrate during a 21-d period of abandoned oral hygiene. Furthermore, no significant change in the percentage of macrophages was reported, although no further description of different macrophage subsets was given (6). Since macrophages play a central role in the cellular and humoral immune response (13, 14), an analysis of different macrophage subsets, rather than an investigation of the whole cell population, is required. The results of the present study indicate that different macrophage subsets are present in clinically healthy gingiva and in the inflammatory infiltrate during experimental gingivitis.

The inflammatory type of macrophage (27E10-positive) shows a significant reduction during the oral hygiene phase and a significant increase during the experimental gingivitis period. Since the 27E10 antigen represents an activated type of macrophage, which is inducible by macrophage-activating factors (IFN-8, TPA and LPS) (29), the appearance of this subset reflects the influx of inflammatory macrophages in response to bacterial accumulation. As the antibody 27E10 defines an early antigen of macrophage differentiation originating from peripheral blood monocytes, 27E10-positive macrophages are preferentially found in the infiltrate.

The number of RM 3/1 cells showed a different progression throughout this study. At the end of the hygiene phase a significant increase, whereas during the experimental gingivitis phase a signifi-

Table 4. Percentage (of the total number of cells counted) of 27E10-positive (inflammatory macrophages), RM 3/1-positive (antiinflammatory macrophages) and 25F9-positive (mature macrophages) cells in the inflammatory infiltrate (IF) and connective tissue (CT) in the three Gingival Index categories

	25F9		27E10		RM 3/1	
	IF	CT	IF	CT	IF	CT
GI 0 n=15	1,1±0,4	0,8±0,3	1,6±0,7	0,7±0,2	6,8±1,8	1,1±0,3
GI 1 n=25	2,1±0,9	1,9±0,5	4,9±1,4	2,4±1,0	2,3±0,9	0,8±0,2
GI 2 n=16	5,0±1,5	2,3±0,8	7,3±1,9	3,2±1,2	1,4±0,6	0,6±0,2

cant decrease, is observed. Since the antigen RM 3/1 is downregulated by macrophage-activating factors, but strongly induced by the corticosteroid, Dexamethasone, RM 3/1-positive macrophages represent an anti-inflammatory cell type (28).

The number of macrophages defined by the monoclonal antibody 25F9 shows no statistically significant difference throughout the study.

The functions of the different macrophage subsets in gingivitis lesions are not yet clear. However, in relation to the Gingival Index scores the appearance and the distribution of the macrophage subpopulations give some evidence of their function.

Because the activated 27E10 phenotype is associated with high Gingival Index scores, this type of macrophage may trigger the immune response. However, it cannot be decided if 27E10 macrophages act as accessory cells by inducing T-helper lymphocytes or as effector cells by exerting cytotoxic effects on bacteria. Seymour *et al.* (6) observed a slight increase in the percentage of T-helper cells during the initial phase of experimental gingivitis.

The increase in the number of RM 3/1-positive cells after the oral hygiene period was associated with a decrease of 27E10 macrophages, without changes in the number of the total macrophage population. Consequently, the increase of 27E10-positive cells during developing gingivitis was associated with a decrease in RM 3/1 macrophages. This might explain the results of two recent experimental gingivitis studies which showed no significant changes in the total number of macrophages throughout a 21-d period of abandoned oral hygiene (3, 6).

Since the increase of RM 3/1 cells was observed during the healing phase, these cells probably are involved in suppressor functions. The 25F9-positive macrophages do not seem to be involved in the inflammatory conditions. They may be involved in the maintenance of a normal steady state.

Since the experimental gingivitis phase was conducted with healthy individuals without evidence of alveolar bone loss or destructive periodontal diseases, the distribution of the three different macrophage subsets represents a "physiological" inflammatory response to microbial plaque accumulation. The result of the present study indicates that the so-called "physiological" gingival inflammation is characterized by the appearance and disappearance of functionally different macrophages. Changes in this pattern may be associated with destructive periodontal lesions.

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TI New human leukocyte clusters of differentiation
AU Stockinger, H.; Majdic, O.; Knapp, W.
SO Transfusion (Bethesda, Md.) (1998), 38(5), 499-505

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TI Shedding of CD163, a novel regulatory mechanism for a member of the scavenger receptor cysteine-rich family.
AU Droste, Anne; Sorg, Clemens; Hoegger, Petra (1)
SO Biochemical and Biophysical Research Communications, (March 5, 1999) Vol. 256, No. 1, pp. 110-113.

TI Identification of the integral membrane protein RM3/1 on human monocytes as a glucocorticoid-inducible member of the scavenger receptor cysteine-rich family (CD163).
AU Hogger P; Dreier J; Droste A; Buck F; Sorg C
SO JOURNAL OF IMMUNOLOGY, (1998 Aug 15) 161 (4) 1883-90.

AU Ritter M; Buechler C; Langmann T; Orso E; Klucken J; Schmitz G
SO PATHOBIOLOGY, (1999) 67 (5-6) 257-61.

TI Regulation of scavenger receptor CD163 expression in human monocytes and macrophages by pro- and antiinflammatory stimuli.
AU Buechler, Christa; Ritter, Mirko; Orso, Evelyn; Langmann, Thomas; Klucken, Jochen; Schmitz, Gerd (1)
SO Journal of Leukocyte Biology, (Jan., 2000) Vol. 67, No. 1, pp. 97-103.

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New human leukocyte clusters of differentiation

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In November 1996, the Sixth International Conference on Human Leukocyte Differentiation Antigens was held in Kobe, Japan, under the chairmanship of Tadamitsu Kishimoto. There, all the data collected from the Sixth International Workshop on Human Leukocyte Differentiation Antigens were summarized and presented. Over a period of approximately 2 years, more than 500 laboratories studied more than 200 leukocyte cell surface molecules with the use of 1152 monoclonal antibodies (MoAbs). As with previous workshops, this international cooperation provided not only a considerable advancement and impact in our understanding of the structure and function of leukocyte surface receptors, but also a number of new clusters of differentiation (CD) designations. In particular, 42 "new" CD entities were established, and several "old," mainly provisional clusters were redefined.

The following table summarizes information on the new CD entities. Because of the good response to the original table, which was published in TRANSFUSION in 1996,[1] we used the same format in the new table. These tables together provide an updated and concise overview on the human CD system.

However, as mentioned above, several old CDs were redefined at the Sixth Conference. Therefore, the original table has to be read with the following revisions: CDw84, CDw90, CDw101, CDw109, CDw116, CDw121a, CDw124, CDw127, and CDw130 lost their "w" (which stands for "workshop," meaning a provisional cluster) and, thus, were promoted to full CDs; CDw65 was split into CD65 and CD65s, and CD40L was renamed CD154; and CD94 was originally thought to be a 43-kDa protein because of the dominant 43-kDa band in ^{125}I-labeled CD94 immunoprecipitates, and, therefore, it was termed KP43. However, it was recently shown that CD94 is a 30-kDa protein poorly labeled by ^{125}I. In parallel, the strongly ^{125}I-labeled 43-kDa protein has been revealed to be identical to NKG2A, whose cDNA has been known for several years. CD94 and NKG2A form a disulfide-linked heterodimer. Both CD94 and NKG2A are members of the Ca^{2+}-dependent (C-type) lectin superfamily of type II transmembrane receptors (see CD23, CD69, CD72, CD161). There is evidence that the CD94/NKG2A complex functions as a receptor for several HLA-A,B,C alleles. NKG2A contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in the cytoplasmic domain, which can interact with the Srchomology 2 domain containing tyrosine phosphatase-1 (SHP-1) and the Src homology 2 domain containing inositol phosphatase (SHIP), both of which are involved in the downregulation of natural killer (NK) cell activity. Thus, the CD94/NKG2A complex may be responsible for the inhibition of a relatively large subset of NK cells. Alternatively, the interaction of CD94 with a less-defined 39-kDa protein seems to form an NK cell receptor that transduces activating signals.

Since the publication of the original table,[1] a number of additional findings in regard to the old CDs have been reported. Examples of some new findings include the identification of CD97 as counterreceptor for CD55; the characterization of CD66 family members CD66a, CD66c, CD66d, and CD66e as neutrophil receptors for internalization of pathogenic *Neisseria* sp. by recognition of opacity-associated outer membrane proteins of the bacteria; and the cloning and characterization of both CD100, a semaphorin (neuronal chemorepellants that direct pioneering neurons during nervous system development) and Ig domain-containing type I transmembrane protein that seems to be involved in T-cell activation and B-cell aggregation and differentiation, and of CD101, a potential T-cell regulatory

ABBREVIATIONS: cADPR = cyclic ADP-ribose; CD = clusters of differentiation; IL = interleukin; ITIM = immunoreceptor tyrosine-based inhibition motif; MoAb(s) = monoclonal antibody(ies); NK = natural killer (cells); PDGF = platelet-derived growth factor; PROW = Protein Reviews On the Web; TNF/NGF = tumor necrosis factor/nerve growth factor; w = "workshop" or provisional cluster. Cell types in {brackets} are those with weak reactivity or heterogeneous expression.

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protein of the Ig supergene family. All new information on the CDs can be obtained from the proceedings of the Sixth Conference and Workshop,[2] which were available at the end of 1997, and the database of the Sixth Workshop, which includes the individual reaction patterns of the analyzed MoAbs. This database can be found in the Internet via <http://mol.genes.nig.ac.jp/hlda.

An excellent resource for quick and convenient access to current information on the CDs is the evolving Protein Reviews On the Web (PROW) database. PROW was established by Stephen Shaw, and it aims to organize and synthesize information on all human proteins and genes. Within this database, the CDs form a subgroup. The individual CD guides of PROW are written and reviewed by many experts and are updated regularly. Links to other resources including Entrez, MEDLINE, and sequence databases are also available. There is free access to PROW via the Internet address <http://www.ncbi.nlm.nih.gov/prow/>.

All those who are awaiting the Seventh Workshop should visit the Internet site <http://gryphon.jr2.ox.ac.uk/hlda7/index.shtml>, where one can register and receive information about organizational developments under the chairmanship of David Mason. The Seventh Workshop will be summed up at the Seventh Conference, June 19-24, 2000, in Harrogate, UK.

CD45RC

Other; Names: Restricted T200; isoform of leukocyte common antigen

Main Reactivity of MoAbs: T-cell subpopulation, B cells, NK cells

Characteristics:

CD45RC antigen: Isoform determinant of CD45 sharing exon 6 (C) sequences. Together with the B determinant, it is contained in the 205-kDa polypeptide. The 220-kDa polypeptide contains the A, B, and C determinants. Probably defines the long-living memory cells within the CD4+ T cells.

Molecular weight: reduced, 220, 205, unreduced, 220, 205

CD65

Other; Names: Desialylated form of CD65s

Main Reactivity of MoAbs: Granulocytes

Characteristics:

CD65 molecule: Carbohydrate structure carried by various glycolipids and glycoproteins. Desialylated form of CD65s (Gal-GlcNAc-Gal-GlcNAc(Fuc)-Gal-GlcNAc-Gal-GlcNAc-Gal-Glc-Cer).

CD65 antibodies: CD65 antibodies are heterogeneous in their cellular-binding characteristics.

CD65s

Other; Names: Ceramide-dodecasaccharide 4c, VIM2 antigen

Main Reactivity of MoAbs: Monocytes, granulocytes, acute myelogenous leukemia blasts

Characteristics:

CD65s molecule: Fucoganglioside with 4 lactosamine repeats, 1 fucose bound to the penultimate N-acetylglucosamine, and 1 terminal α2,3 linked N-acetyl-neuraminic acid(NeuAc-Gal-GlcNAc-Gal-GlcNAc(Fuc)-Gal-GlcNAc-Gal-GlcNAc-Gal-Glc-Cer). It is based on a type II chain (Galβ1-4GlcNAcβ1-3Galβ1-R). Carried by various glycolipids and glycoproteins. Potential ligand for CD62E and CD62L.

CD65s antibodies: Some induce oxidative burst and inhibit phagocytosis.

CD66f

Other; Names: Pregnancy-specific glycoprotein (PSG), SP-1

Main Reactivity of MoAbs: Myeloid cells, placenta, fetal liver

Characteristics:

CD66f molecule: The CD66f molecule is a secreted molecule. Member of the carcinoembryonic antigen (CEA) gene family. Consists of one IgV-like domain and two to three IgC-like domains. There are existing many splice variants from 11 genes. The function is still unclear. However, low blood level predicts spontaneous abortion.

Molecular weight: reduced, 54-72

CD114

Other; Names: Granulocyte–colony-stimulating factor (G–CSF) receptor

Main Reactivity of MoAbs: Granulocytes, monocytes

Characteristics:

CD114 molecule: The CD114 molecule is a type I transmembrane glycoprotein. Member of the cytokine receptor family. The extracellular domain consists of an Ig-like domain, a cytokine receptor domain, and four fibronectin type III domains, the first of which contains the WSXWS motif.

Molecular weight: reduced, 130

CDw123

Other; Names: Interleukin (IL) 3 receptor α chain

Main Reactivity of MoAbs: Monocytes

Characteristics:

CDw123 molecule: Type I integral membrane protein belonging to the cytokine receptor (hematopoietin) superfamily. The extracellular region consists of an N-terminal domain (similar to that found in the α-chain of CD116 and CDw125), followed by a cytokine receptor domain and fibronectin type III domain, which contains a WSXWS motif. Associates with CDw131 (common to CD116 and CDw125) to form the functional IL-3 receptor.

Molecular weight: reduced, 70

CDw125

Other; Names: IL-5 receptor α chain

Main Reactivity of MoAbs: Eosinophils, basophils, activated B cells

Characteristics:

CDw125 molecule: Type I integral membrane protein belonging to the cytokine receptor (hematopoietin) superfamily. The extracellular region consists of an N-terminal domain (similar to that found in the α-chain of CD116 and CDw123), followed by a cytokine receptor domain and a WSXWS motif-containing fibronectin type III domain. Associates with CDw131 (common to CD116 and CDw123) to form the IL-5 receptor.

Molecular weight: reduced, 60

CDw131

Other; Names: Common β-chain

Main Reactivity of MoAbs: myeloid cells, pre-B cells

Characteristics:

CDw131 molecule: The CDw131 molecule is a member of the cytokine receptor superfamily. It is termed common β-chain, because it associates with either the CD116, CDw123, or CDw125 molecule to form the appropriate functional receptor.

Molecular weight: reduced, 120

CD132

Other; Names: Common γ-chain

Main Reactivity of MoAbs: Leukocytes broad

Characteristics:

CD132 molecule: The CD132 molecule is a member of the cytokine receptor superfamily. It functions as γ-chain of the receptors for IL-2 (see CD25 and CDw122), IL-4 (see CD124), IL-7 (see CD127), IL-9, and IL-15 (see also CDw122). Mutation causes X-linked severe combined immunodeficiency.

Molecular weight: reduced, 64

CD134

Other; Names: OX40

Main Reactivity of MoAbs: Activated T-cell subpopulation

Characteristics:

CD134 molecule: Type I integral membrane protein. Member of the tumor necrosis factor/nerve growth factor (TNF/NGF) receptor family (see CD27, CD30, CD40, CD95, CD120a, CD120b, CDw137). The extracellular region consists of three cysteine-rich repeats. Costimulatory molecule of T cells. Involved in T- and B-cell and T-cell and endothelial cell interactions by binding to the OX40 ligand.

Molecular weight: reduced, 48-50

CD135

Other; Names: FMS-like tyrosine kinase 3 (FLT 3); fetal liver kinase 2 (flk 2); stem cell kinase-1 (STK-1)

Main Reactivity of MoAbs: Progenitor cell subpopulation, pre-B cells

Characteristics:

CD135 molecule: Type I integral membrane protein, belonging to the Ig and receptor tyrosine kinase family (see CD115, CD117, CD140a, CD140b). The extracellular region consists of five Ig-like domains. Receptor for flt3 ligand. Important receptor and ligand system for hemopoietic cell proliferation and differentiation.

Molecular weight: reduced, 155; unreduced, 130

CDw136

Other; Names: Macrophage-stimulating protein receptor, RON

Main Reactivity of MoAbs: Monocytes, granulocytes, epithelial cells

Characteristics:

CDw136 molecule: Member of the hepatocyte growth factor receptor subgroup of the receptor tyrosine kinase family. The mature protein is organized as a disulfide-linked α/β dimer, which is derived from a single-chain pro RON. The α-chain (35-kDa) is bound extracellularly to the transmembrane β-chain (150-kDa). Transfers apoptotic as well as growth signals. Involved in the regulation of production of blood cells and in the development of epithelial tissue.

Molecular weight: reduced, 35, 150; unreduced, 185

CDw137

Other; Names: 4-1BB

Main Reactivity of MoAbs: Activated T cells

Characteristics:

CDw137 molecule: Type I integral membrane protein that forms disulfide-linked homodimers. Member of the TNF/NGF receptor family (see CD27, CD30, CD40, CD95, CD120a, CD120b, CD134). The extracellular region consists of three cysteine-rich repeats. Receptor for 4-1BB ligand which belongs to the TNF family. Seems to be important for T-cell survival.

Molecular weight: reduced, 30; unreduced, 80

CD138

Other; Names: Syndecan-1, B-B4

Main Reactivity of MoAbs: Plasma cells, epithelial cells

Characteristics:

CD138 molecule: Type I integral membrane protein. The extracellular domain bears heparan sulfate glycosaminoglycans through which it binds both growth factors and extracellular matrix constituents, including basic fibroblast growth factor, collagens, thrombospondin, and fibronectin. The transmembrane and cytoplasmic domains are highly conserved. The cytoplasmic domain interacts with cytoskeletal components. Proposed to have roles in growth fac-

tor action, extracellular matrix adhesion, and cytoskeletal organization that controls cell morphology.
Molecular weight: reduced, 80-160

CD139

Other; Names:
Main Reactivity of MoAbs: B cells, monocytes, granulocytes
Characteristics:
Not fully characterized, defined by MoAbs CAT13.4G9 and BU30.
Molecular weight: reduced, 220, 250; unreduced, 228

CD140a

Other; Names: α-type platelet-derived growth factor (PDGF) receptor
Main Reactivity of MoAbs: Undetectably expressed
Characteristics:
CD140a molecule: The CD140a molecule is the α-type PDGF receptor. Type I integral membrane protein belonging to the Ig and receptor tyrosine kinase family (see CD115, CD117, CD135, CD140b). The extracellular region consists of five Ig-like domains. Related to the CD140b molecule. PDGF binding induces either homodimerization or heterodimerization with the CD140b molecule. Binds both PDGF-A and PDGF-B chains. Induces a mitogenic response.
Molecular weight: reduced, 180

CD140b

Other; Names: β-type PDGF receptor
Main Reactivity of MoAbs: Focal endothelial cells, stromal cell lines
Characteristics:
CD140b molecule: The CD140b molecule is the β-type PDGF receptor. Type I integral membrane protein belonging to the Ig and receptor tyrosine kinase family (see CD115, CD117, CD135, CD140a). The extracellular region consists of five Ig-like domains. Related to the CD140a molecule. PDGF binding induces either homodimerization or heterdimerization with the CD140a molecule. Binds PDGF-B chains. Induces a mitogenic response, actin reorganization, and chemotaxis.
Molecular weight: reduced, 180

CD141

Other; Names: Thrombomodulin
Main Reactivity of MoAbs: Myeloid cells, platelets, endothelial cells
Characteristics:
CD141 molecule: Type I transmembrane protein. The extracellular region consists of an N-terminal C-type lectin domain followed by six epidermal growth factor-like repeats. Receptor for thrombin. Receptor-bound thrombin activates protein C but also plasma pro-carboxypeptidase B (thrombin-activatable fibrinolysis inhibitor), resulting in initiation of the protein C-dependent anticoagulant pathway and or inhibition of fibrinolysis, respectively.
Molecular weight: reduced, 100; unreduced, 75

CD142

Other; Names: Tissue factor, thromboplastin, coagulation factor III
Main Reactivity of MoAbs: Activated monocytes, activated endothelial cells, epithelial cells
Characteristics:
CD142 molecule: Type I transmembrane protein. Member of the class II cytokine receptor family. Related to the interferon (IFN)-α receptor and the CDw119 molecule. The extracellular region contains two fibronectin type III domains. High-affinity receptor for the serine protease factor VII, and it is the essential cofactor for factor VII. Forms a ternary complex with factors VII and X to induce the extrinsic pathway of the coagulation cascade. The gene is located on chromosome 1p21-p22.
Molecular weight: reduced, 45; unreduced, 45

CD143

Other; Names: Angiotensin-converting enzyme (ACE), peptidyl dipeptidase A, EC3.4.15.1
Main Reactivity of MoAbs: Endothelial cells, epithelial cells
Characteristics:
CD143 molecule: The CD143 molecule is the somatic form of angiotensin-converting enzyme. It is a zinc metallopeptidase with substrate specificity for the vasoactive peptides angiotensin I and bradykinin. The extracellular region is organized in two catalytic domains. It is differentially expressed along the vascular tree. Endothelial cells of arterioles, small arteries, and capillaries in organs such as lung are CD143 positive. Endothelial cells of large arteries and veins are mostly negative. Germinal center cells express a lower molecular form lacking the N-terminal catalytic domain in the extracellular region. The gene is located on chromosome 17q23.
Molecular weight: reduced, 170; unreduced, 170

CD144

Other; Names: Vascular endothelial cell cadherin; cadherin 5; 7B4
Main Reactivity of MoAbs: Endothelial cells
Characteristics:
CD144 molecule: Type I transmembrane protein. The extracellular region consists of five cadherin-homologous repeats. It is a Ca^{2+}-dependent homophilic cell adhesion molecule. It interacts with catenins, which link CD144 to the cytoskeleton; this complex is crucial for the regulation of cell adhesion.
Molecular weight: reduced, 130, unreduced, 135

CDw145

Other; Names:
Main Reactivity of MoAbs: Endothelial cells
Characteristics:
Not fully characterized. Defined by the MoAbs 7E9 and P7A5.
Molecular weight: reduced, 25

CD146

Other; Names: Muc18, S-endo; melanoma cell adhesion molecule (MCAM or Mel-CAM)
Main Reactivity of MoAbs: Endothelial cells, melanoma cells, activated T-cell subpopulation, smooth muscle cells
Characteristics:
CD146 molecule: Type I transmembrane protein. Member of the Ig supergene family. The extracellular region consists of five Ig-like domains (two of theV-type and three of the C2-type). Potential cell adhesion molecule.
Molecular weight: reduced, 130; unreduced, 118

CD147

Other; Names: Neurothelin, basigin, M6, EMMPRIN
Main Reactivity of MoAbs: Leukocytes broad, endothelial cells, platelets, red cells
Characteristics:
CD147 molecule: Type I transmembrane protein. Member of the Ig supergene family. The extracellular region consists of two Ig-like domains of the C2 type. It bears the high-frequency blood group antigen OK^a. Potential cell adhesion molecule that, upon interaction with fibroblasts, may induce collagenases in these cells. The gene is located on chromosome 19p13.3.
Molecular weight: reduced, 50-60; unreduced, 45-55
CD147 antibodies: Some MoAbs seem to recognize epitopes that are mainly restricted to monocytes, endothelial cells, and activated T cells.

CD148

Other; Names: p260; human protein tyrosine phosphatase (HPTPη), density-enhanced protein tyrosine phosphatase-1
Main Reactivity of MoAbs: Granulocytes, monocytes, T cells, dendritic cells, platelets
Characteristics:
CD148 molecule: Type I transmembrane protein. It is a receptor protein tyrosine phosphatase of type III; this type is characterized by the exclusive presence of fibronectin type III motifs in the extracellular domain. The gene is located on chromosome 11p11.2.
Molecular weight: reduced, 200-260; unreduced, 200-260

CDw149

Other; Names: MEM-133
Main Reactivity of MoAbs: Lymphocytes, monocytes, {granulocytes, platelets}
Characteristics: Not fully characterized. Defined by MoAbs MEM-120 and MEM-133.
Molecular weight: reduced, 120

CDw150

Other; Names: Signaling lymphocytic activation molecule (SLAM), IPO-3
Main Reactivity of MoAbs: T- and B-cell subpopulations, thymocytes
Characteristics:
CDw150 molecule: Type I transmembrane protein, member of the Ig supergene family. The extracellular region consists of one V-like and one C-like Ig domain. Three further isoforms have been described: a variant membrane form with a truncated cytoplasmic domain, a soluble secreted form lacking 30 amino acids encompassing the entire transmembrane domain, and a cytoplasmic form lacking the leader sequence. It is a homotypic adhesion molecule involved in T- and T-cell, T- and B-cell, and B- and B-cell interactions and thereby in the regulation of T-cell and B-cell activation.
Molecular weight: reduced, 70
CDw150 antibodies: Induce T-cell stimulation in a CD28-independent, IL-2-independent, cyclosporin A-sensitive manner.

CD151

Other; Names: Platelet-endothelial tetraspan antigen-3
Main Reactivity of MoAbs: Endothelial cells, platelets, stromal cells
Characteristics:
CD151 molecule: The CD151 molecule is a member of the tetraspan family (see CD9). Part of the signaling complex of FcγRIIa and associated with β1 integrins.
Molecular weight: reduced, 27
CD151 antibodies: Induce homotypic adhesion of HEL and K562 cells and aggregation of platelets.

CD152

Other; Names: Cytotoxic T-lymphocyte antigen-4
Main Reactivity of MoAbs: Activated T cells
Characteristics:
CD152 molecule: Type I integral membrane protein. Member of the Ig supergene family. The extracellular region consists of one IgV-like domain. It is expressed as disulfide-linked homodimer. Receptor for CD80 and CD86. It functions as a regulator of T-cell activation.
Molecular weight: reduced, 44; unreduced, 80

CD153

Other; Names: CD30 ligand
Main Reactivity of MoAbs: Activated T cells

Characteristics:
CD153 molecule: Type II transmembrane protein, member of the TNF family (see CD70, CD95L, CD154). Counterreceptor for CD30.
Molecular weight: reduced, 40

CD154

Other; Names: gp39; TNF-related activation protein (TRAP)-1, T cell- and B cell-activating molecule (T-BAM); CD40 ligand
Main Reactivity of MoAbs: Activated T cells, mast cells
Characteristics:
CD154 molecule: Type II integral membrane protein belonging to the TNF family (see CD70, CD95L, CD153). It is essential for germinal center formation and for Ig class switching. Nonfunctional or defective expression due to point mutations causes hyper-IgM syndrome.
Molecular weight: reduced, 39; unreduced, 39

CD155

Other; Names: Polio virus receptor
Main Reactivity of MoAbs: Monocytes, CD34+ cells
Characteristics:
CD155 molecule: Type I transmembrane glycoprotein. Member of the Ig supergene family. The extracellular region consists of three Ig-like domains (one of the V-type and two of the C-type). The gene is located on chromosome 19q13.1-13.2.
Molecular weight: reduced, 80-90

CD156

Other; Names: MS2, ADAM-8
Main Reactivity of MoAbs: Monocytes, granulocytes
Characteristics:
CD156 molecule: The CD156 molecule belongs to the ADAM (a disintegrin and metalloproteinase domain) family, which comprises about 25 members. The extracellular region consists of a disintegrin-, metalloproteinase-, cysteine rich-domain and an epidermal growth factor domain. Probably involved in loosening of cell-cell, cell-matrix contacts. The gene is located on chromosome 10q26.1-26.3.
Molecular weight: reduced, 69

CD157

Other; Names: Bone marrow stromal cell antigen-1 (Bst-1); Mo5
Main Reactivity of MoAbs: Monocytes, granulocytes, bone marrow stroma cells
Characteristics:
CD157 molecule: The CD157 molecule is glycosylphosphatidylinositol-anchored. It is a CD38-like protein having both ADP-ribosyl cyclase activity and cyclic ADP-ribose (cADPR) hydrolase activity. cADPR is a stimulator of Ca^{2+} release from intracellular pools. The gene is located on chromosome 4p15.
Molecular weight: reduced, 42-50; unreduced, 42-50

CD158a

Other; Names: p58.1/p50.1
Main Reactivity of MoAbs: NK cell and T-cell subpopulations
Characteristics:
CD158a molecule: Type I integral membrane protein. p58.1 belongs to the killer cell inhibitory receptors of the Ig supergene family. The cytoplasmic region of p58.1 contains two ITIM motifs. In contrast, p50.1 has activating function; it is linked to a different transmembrane region that contains a charged residue (lysine) and a shorter cytoplasmic region lacking both ITIM motifs. The extracellular region of both molecules is identical and consists of two Ig-like domains. Receptor for HLA CW2, CW4, CW5, CW6. The gene is located on chromosome 19.
Molecular weight: reduced, 58/50; unreduced, 58/50

CD158b

Other; Names: p58.2/p50.2
Main Reactivity of MoAbs: NK cells
Characteristics:
CD158b molecule: Type I integral membrane protein. p58.2 belongs to the killer cell inhibitory receptors of the Ig supergene family. The cytoplasmic region of p58.2 contains two ITIM motifs. In contrast, p50.2 has activating function; it has a shorter cytoplasmic region lacking both ITIM motifs. The extracellular region of both molecules is identical and consists of two Ig-like domains. Receptor for HLA CW1, CW3, CW7, CW8.
Molecular weight: reduced, 58/50; unreduced, 58/50

CD161

Other; Names: NKR-P1A
Main Reactivity of MoAbs: NK cells, monocytes, T-cell subpopulation, thymocyte precursors
CD161 molecule: Disulfide-linked homodimer. Member of the Ca^{2+}-dependent (C-type) lectin superfamily of type II transmembrane receptors (see CD23, CD69, CD72, CD94). The gene is located on chromosome 12.
Molecular weight: reduced, 30; unreduced, 60

CD162

Other; Names: P-selectin glycoprotein ligand (PSGL-1), CD62 ligand
Main Reactivity of MoAbs: Monocytes, granulocytes, T cells
Characteristics:
CD162 molecule: The CD162 molecule is expressed as a disulfide-linked homodimer. The gene is located on chromosome 12q24. Counterreceptor of CD62E, CD62L and CD62P. Involved in leukocyte roling.

Molecular weight: reduced, 110; unreduced, 220
CD162 antibodies: MoAb PL1 is a blocking MoAb.

CD163

Other; Names: KiM8, M130, GHI/61
Main Reactivity of MoAbs: Monocytes
Characteristics:
CD163 molecule: The CD163 molecule is a type I transmembrane molecule. The extracellular domain consists of nine scavenger-like repeating elements.
Molecular weight: reduced, 130

CD164

Other; Names: Multi-glycosylated core [protein 24] (MGC)-24, MUC-24
Main Reactivity of MoAbs: T cells, monocytes, granulocytes, epithelial cells, stroma cells, bone marrow cells
Characteristics:
CD164 molecule: The CD164 molecule forms disulfide-linked homodimers. It is a sialomucin-like glycoprotein (see CD34, CD43, CD68). Involved in adhesion of hemopoietic cells to stroma cells. The gene is located on chromosome 6q21.
Molecular weight: reduced, 80; unreduced, 160

CD165

Other; Names: AD2, gp37
Main Reactivity of MoAbs: Platelets, NK subpopulation, T-cell subpopulation, monocytes, immature thymocytes
Characteristics:
CD165 molecule: Not fully characterized. Defined by the MoAbs AD2 and SN2.
Molecular weight: reduced, 42; unreduced, 37

CD166

Other; Names: Activated leukocyte-cell adhesion molecule, CD6 ligand
Main Reactivity of MoAbs: Activated monocytes, activated T cells, thymus epithelium, neurons
Characteristics:
CD166 molecule: Member of the Ig supergene family. The extracelluar region consists of four Ig-like domains. Counterreceptor for CD6. The gene is located on chromosome 3q13.1-13.2.
Molecular weight: reduced, 100; unreduced, 100

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